AUC 2019

24th International Analytical Ultracentrifugation Workshop and Symposium

University of Canterbury, Christchurch, New Zealand

24th – 29th August 2019

UC
UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

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We are extremely grateful to the following for their generous sponsorship of AUC 2019.

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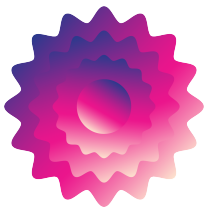

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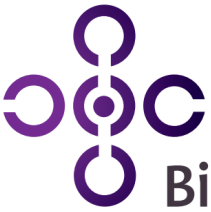


Biomolecular Interaction Centre

Kia ora!

On behalf of the Scientific Organising Committee (SOC) and the Local Organising Committee (LOC) I would like to welcome you to the University of Canterbury and to AUC2019, the 24rd International AUC Workshop and Symposium. This booklet contains all the information we think you will need for a successful meeting and an enjoyable visit, including details of the venue, workshop and symposium programmes, catering and social activities. The abstracts for both oral and poster presentations are also presented at the back of this booklet.

If you need any help with anything, please don't hesitate to ask a member of the LOC - see below for details.

I wish you a very enjoyable stay in our lovely city and a terrific meeting. Thanks for coming!

All the best,
Ren Dobson, Chair AUC2019

Local Organising Committee (LOC)

If you need some help out of hours, don't hesitate to call one of us - start with Ren!

Ren Dobson	+ 64 21 265 7750
Grant Pearce	+ 64 27 634 7072
David Goldstone	
Michael Griffin	
Tatiana Soares da Costa	
Chris Horne	

Scientific Organising Committee (SOC)

Olwyn Byron	University of Glasgow, UK
Helmut Cölfen	Universität Konstanz, Germany
Borries Demeler	University of Lethbridge, Canada
Renwick Dobson	University of Canterbury, New Zealand
Karen Fleming	Johns Hopkins University, US
Phillip Koldeway	F. Hoffmann-La Roche Ltd, Basel, Switzerland
Luitgard Nagel-Steger	Heinrich Heine Universität Düsseldorf, Germany
Trushar Patel	University of Lethbridge, Canada
Alexandra Solovyova	Newcastle University, UK
Walter Stafford	Harvard Medical School, US
Susumu Uchiyama	Osaka University, Japan

Registration

Registration for the Workshops is 12:00-13:00 Saturday August 24 in Engineering Core, next to room E5.
Registration for the Symposium is 17:00-19:00 Tuesday in Engineering Core, next to room E5.
Please refer to maps on the next page.

Car Parking

Visitors to campus are able to park in designated visitor parking areas. To park in these areas visitors will need to display an $8 all day parking coupon or a parking coupon obtained from a pay-and-display unit.

Getting to UC

The University of Canterbury has buses running every few minutes from plenty of stops around campus. MetroCards are free electronic pre-pay discount cards for use on Christchurch buses, and can be purchased from the UBS campus bookstore. You save at least 25% on the cash fare, and you are entitled to unlimited number of transfers within two hours. Bus routes servicing campus include:

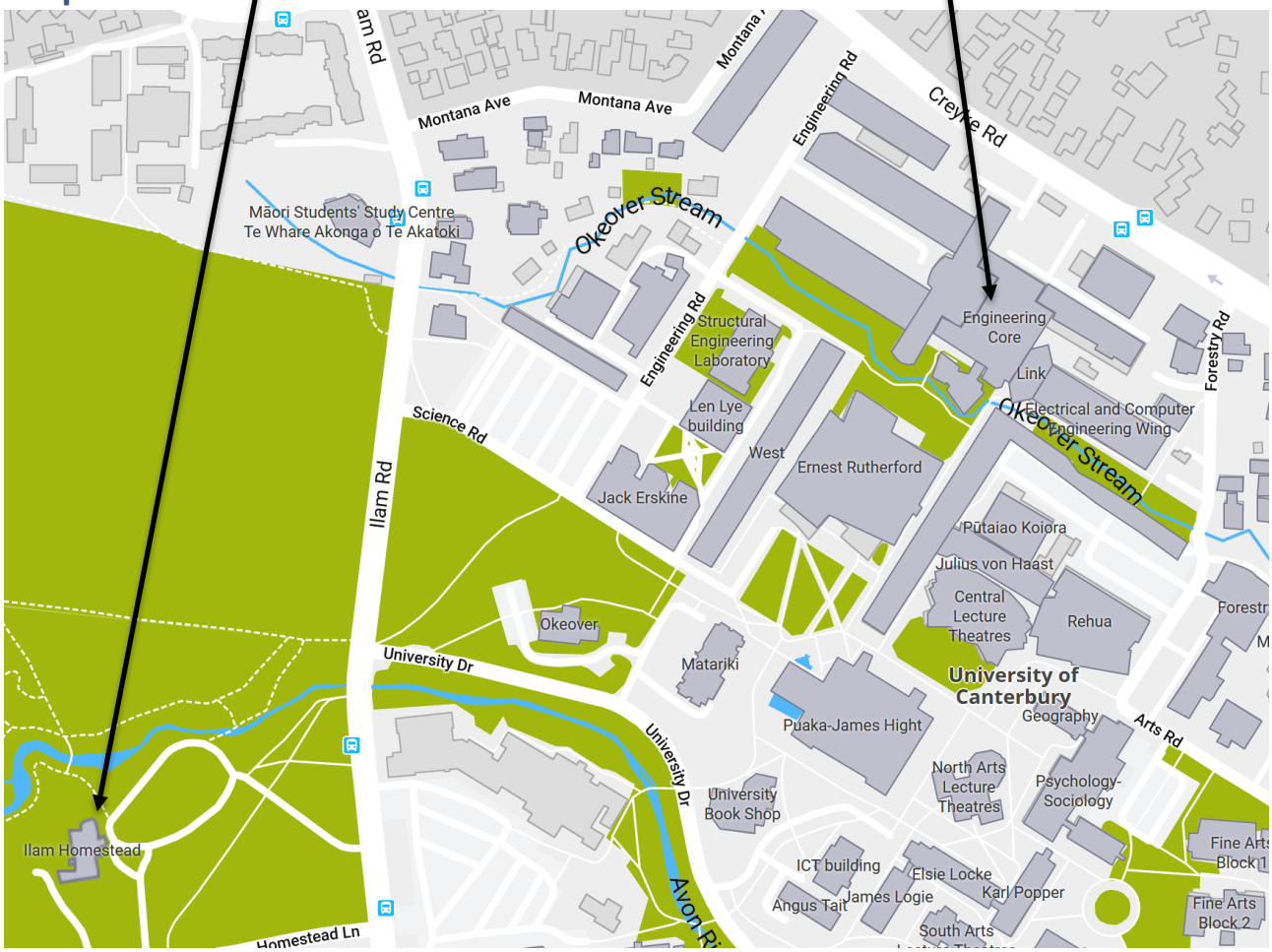
- Purple Line: Airport - Sumner via Avonhead stops on Ilam Rd
- 120 Burnside - Spreydon stops on Clyde Rd
- 130 Hei Hei - Avonhead - stops on Creyke Rd
- 100 Wigram - The Palms - stops on Clyde Rd, Creyke Rd and Ilam Rd

Alternatively, both Lime and Beam eScooters are available in the city.

Venues

The workshop and symposium will be held in the Engineering Core. All meals, excluding breakfast, are provided with registration. Tea, coffee and lunch will be served in the Engineering Core. Dinner will be served at the Ilam Homestead.

Map



(https://www.canterbury.ac.nz/maps/home)

Wi-Fi

eduroam Wi-Fi for visitors

If you are visiting the University of Canterbury from another institution that uses eduroam Wi-Fi, you can also use it here. Just make sure your device is set up before you arrive. eduroam is available at numerous Wi-Fi hotspots. Please use eduroam if you can.

Configuration

- SSID: **eduroam**
- Network access method: **IEEE 802.1x**
- Use your **home institution** username and password

UCVistor Wi-Fi

Alternatively, you can logon to the UCvisitor network

- **Guest Username: uc4273**
- **Guest Password: 191370**

Emergencies

111 – The main emergency number

This is the emergency number for police, ambulance, and fire brigade. Calling is free, and you can call from a locked mobile phone, or if you have no phone credit.

105 – The non-emergency medical number

Dial this number for illnesses and minor injuries where life isn't threatened, but you would like some advice on what to do next. Calls are free.

Official language

The workshop and symposium language is English.

Smoking

In accordance with University policy smoking is not permitted in anywhere on campus.

Posters

There will be one Poster Session from 1730-1900 on Wednesday 28 August

Please place posters on boards according to assigned poster number (see Poster Abstracts below) as soon as you can and by no later than 17:00 on Wednesday 28 August. Velcro and pins will be provided. Please take posters down by the end of afternoon break on Thursday 29 August.

Symposium speakers

Assistance for loading talks will be provided by the LOC. Please load your talk onto the desired laptop during the break immediately prior to the commencement of the session in which you are speaking. Please assess compatibility of laptops used well in advance of the relevant session. Note that power adaptors for laptops are not provided, but a laser pointer will be available.

Workshop programme

	Workshop leader(s)	Title	Sessions
01	Tom Laue	Introduction to AUC: Simple AUC theory and practices	1, Repeat: 3
02	Tom Laue	Introduction to AUC: Optical systems	2, Repeat 4
03	Sandy Ross & Harmen Steele	Fluorescence Spectroscopy: a complement to AUC	1, Repeat: 6
04	Christine Ebel & Karen Fleming	Membrane Proteins	1, Repeat: 2
05	Emre Brookes	US-SOMO Hydrodynamic Modelling	1-3, Repeat: 7-9
06	Emre Brookes	US-SOMO Small Angle Scattering	4-6
07	Steve Perkins & Emre Brookes	SASSIE2	7-10
08	Helmut Cölfen	Nanoparticle Analysis by AUC	2, Repeat: 4
09	Borries Demeler	Introduction to UltraScan	3-6
10	Borries Demeler	AUC Problem Solving with UltraScan	7-10
11	Walter Stafford & Jack Correia	SEDANAL	3-10
12	John Philo	SEDNTERP	5
13	John Philo	Time-derivative Analysis with the Program DCDT+	7, Repeat 8
14	John Philo	Getting Confidence Limits Using the Program SVEDBERG	9, Repeat 10
15	Ivo Nischang & Igor Perevyazko	Hydrodynamic analysis of synthetic and bio-macromolecules	5-6, Repeat: 9-10
16	Chad Brautigam	GUSSI	3
17	Chad Brautigam	Membrane proteins and conformational changes	5-6
18	Chad Brautigam	Multisignal SV and Analyzing SE Data	7-8
19	Chad Brautigam	Complementary Techniques: ITC and MST	9-10
20	Johannes Walter	HDR-MULTIFIT–Analysis of turbidity data and determination of particle size distributions	5-6

Saturday 24th August			
09:00-12:00	Arrival and check-in		
12:00-12:45	Workshop registration and lunch		
12:45-13:00	Welcome and housekeeping in rm E5		
13:00-15:00	Workshop session 1		
	101	**Introduction to AUC: Simple AUC theory and practices:** *Tom Laue*	E5 (17)
	105	**US-SOMO hydrodynamic modelling 1**: *Emre Brookes*	E7 (16)
	104	**Membrane proteins**: *Christine Ebel & Karen Fleming*	129 (10)
	103	**Fluorescence spectroscopy: a complement to AUC**: *Sandy Ross & Harmen Steele*	E6 (18)
15:00-15:30	Afternoon tea		
15:30-17:30	Workshop session 2		
	102	**Introduction to AUC: Optical systems**: *Tom Laue*	E5 (14)
	105	**US-SOMO hydrodynamic modelling 2**: *Emre Brookes*	E7 (16)
	104	**Membrane proteins (repeat)**: *Christine Ebel & Karen Fleming*	129 (13)
	108	**Nanoparticle analysis by AUC**: *Helmut Cölfen*	E6 (19)
17:30-20:00	**Drinks and Beckman Coulter Welcome Dinner** **UC Staff Club (Fush catering)**		

Sunday 25th August			
09:00-11:00	Workshop session 3		
	209	**Introduction to UltraScan 1**: *Borries Demeler*	E6 (15)
	205	**US-SOMO Hydrodynamic Modelling 3**: *Emre Brookes*	E7 (16)
	211	**SEDANAL—Introduction and sed. vel. of noninteracting and interacting systems 1**: *Walter Stafford & Jack Correia*	128 (13)
	201	**Introduction to AUC: Simple AUC theory and practices (repeat)**: *Tom Laue*	129 (11)
	216	**GUSSI**: *Chad Brautigam*	E5 (16)
11:00-11:30	Morning coffee		
11:30-13:30	Workshop session 4		
	209	**Introduction to UltraScan 2**: *Borries Demeler*	E6 (15)
	206	**US-SOMO small angle scattering 1**: *Emre Brookes*	E7 (15)
	211	**SEDANAL—Introduction and sed. vel. of noninteracting and interacting systems 2**: *Walter Stafford & Jack Correia*	128 (13)
	202	**Introduction to AUC: Optical systems (repeat)**: *Tom Laue*	129 (11)
	208	**Nanoparticle analysis by AUC (repeat)**: *Helmut Cölfen*	E5 (13)
13:30-14:30	Lunch		
14:30-16:30	Workshop session 5		
	209	**Introduction to UltraScan 3**: *Borries Demeler*	E6 (15)
	206	**US-SOMO small angle scattering 2** *Emre Brookes*	E7 (15)
	211	**SEDANAL—Sedimentation velocity of interacting systems 1**: *Walter Stafford & Jack Correia*	128 (13)
	217	**Membrane proteins and conformational changes 1**: *Chad Brautigam*	E5 (22)
	212	**SEDNTERP**: *John Philo*	129 (12)
	215	**Hydrodynamic analysis of synthetic and bio-macromolecules 1**: *Ivo Nischang & Igor Perevyazko*	E12 (14)
	220	**HDR-MULTIFIT–Analysis of turbidity data and determination of particle size distributions 1**: *Johannes Walter*	E13 (12)
16:30-17:00	Afternoon tea		
17:00-19:00	Workshop session 6		
	209	**Introduction to UltraScan 4**: *Borries Demeler*	E6 (15)
	206	**US-SOMO small angle scattering 1**: *Emre Brookes*	E7 (15)
	211	**SEDANAL—Sedimentation velocity of interacting systems 2**: *Walter Stafford & Jack Correia*	128 (13)
	217	**Membrane proteins and conformational changes 2**: *Chad Brautigam*	E5 (22)
	203	**Fluorescence spectroscopy: a complement to AUC (repeat)**: *Sandy Ross & Harmen Steele*	129 (7)
	215	**Hydrodynamic analysis of synthetic and bio-macromolecules 1**: *Ivo Nischang & Igor Perevyazko*	E12 (14)
	220	**HDR-MULTIFIT–Analysis of turbidity data and determination of particle size distributions 2**: *Johannes Walter*	E13 (12)
19:00-21:00	**Dinner** **UC Staff Club (Pizza catering)**		

Monday 26th August			
09:00-11:00	Workshop session 7		
	309	**AUC problem solving with UltraScan 1**: *Borries Demeler*	E6 (13)
	305	**US-SOMO hydrodynamic modelling 1 (repeat)**: *Emre Brookes*	E7 (9)
	311	**SEDANAL—Sedimentation velocity of non-ideal systems**: *Walter Stafford & Jack Correia*	128(13)
	318	**Multisignal SV and analyzing SE Data 1**: *Chad Brautigam*	E5 (24)
	307	**SASSIE2 1**: *Steve Perkins*	129 (9)
	313	**Time-derivative analysis with the program DCDT+**: *John Philo*	E12 (9)
11:00-11:30	Morning coffee		
11:30-13:30	Workshop session 8		
	309	**AUC problem solving with UltraScan 2**: *Borries Demeler*	E6 (13)
	305	**US-SOMO hydrodynamic modelling 2 (repeat)**: *Emre Brookes*	E7 (9)
	311	**SEDANAL—Sedimentation equilibrium of non-interacting and interacting systems**: *Walter Stafford & Jack Correia*	128 (13)
	318	**Multisignal SV and analyzing SE Data 2**: *Chad Brautigam*	E5 (24)
	307	**SASSIE2 2**: *Steve Perkins*	129 (9)
	313	**Time-derivative analysis with the program DCDT+ (repeat)**: *John Philo*	ER 260 (4)
13:30-14:30	Lunch		
14:30-16:30	Workshop session 9		
	319	**AUC problem solving with UltraScan 3**: *Borries Demeler*	E6 (13)
	305	**US-SOMO hydrodynamic modelling 3 (repeat)**: *Emre Brookes*	E7 (9)
	311	**SEDANAL—Advanced topics: multiwavelength analysis**: *Walter Stafford & Jack Correia*	128 (13)
	319	**Complementary Techniques: ITC and MST 1**: *Chad Brautigam*	E5 (22)
	307	**SASSIE2 3**: *Steve Perkins*	129 (9)
	314	**Getting Confidence Limits Using the Program SVEDBERG**: *John Philo*	E14 (9)
	315	**Hydrodynamic analysis of synthetic and bio-macromolecules 1 (repeat)**: *Ivo Nischang & Igor Perevyazko*	E12 (6)
16:30-17:00	Afternoon tea		
17:00-19:00	Workshop session 10		
	309	**AUC problem solving with UltraScan 4**: *Borries Demeler*	E6 (13)
	307	**SASSIE2 4**: *Emre Brookes & Steve Perkins*	129 (9)
	311	**SEDANAL—Advanced topics: multiwavelength Analysis**: *Walter Stafford & Jack Correia*	128 (13)
	319	**Complementary techniques: ITC and MST 1**: *Chad Brautigam*	E5 (22)
	314	**Getting confidence limits using the program SVEDBERG (repeat)**: *John Philo*	119 (6)
	315	**Hydrodynamic analysis of synthetic and bio-macromolecules 2 (repeat)**: *Ivo Nischang & Igor Perevyazko*	E12 (6)
19:00-21:00	**Dinner** **UC Staff Club (Burger cantering)**		

Excursion

Tuesday 27th August – A Day out in the Waipara Region

In Summary - Two tours - one each pre and post lunch and a very relaxed pass around and food station style lunch at Black Estate Winery

Limestone Hills – One of our tours today is to a small farm in the Waipara Valley where Gareth and Camille Renowden grow four kinds of gourmet truffles, and pinot noir and Syrah grapes. Limestone Hills is the only truffière in New Zealand producing four species of truffles.

Black Estate - has three organic hillside vineyards on clay limestone soils in North Canterbury. You will be visiting their Cellar door and architecturally designed wintery for lunch and having a tour of their Netherwood Block with its Pinot Noir and Chardonnay grapes.

What to bring:
Cash/Card for wine purchase and drinks at the winery for your lunch.
Walking shoes and we will be outside in an NZ winter so could be pretty chilly! We will have a wet weather contingency.

Renwick Dobson +64 21 265 7750
Emma the Event planner +64 21 678 877

8.30 am	Delegates Meet at Bus Stop at University and ticked off lists onto two buses	Delegates to Creyke Road Bus Stop outside Engineering block where conference is being held. Look out for Ren and two "Ritchie's" buses. PLEASE don't be late!
9.00 am	Depart Christchurch	Welcome on the Bus and chat about the day – 1 Bus to each tour
10.15 am	Buses arrive at either: Limestone Hills Truffles Back Estate Netherwood Vineyard	Please note Bathrooms at each location and easy walk from the bus to the tour. Tours are each 1.5 hours.
10.15 am	Tours commence	Tour Limestone Hills and Truffles Gareth Tour Black Estate with Nicolas
12.00 pm	Everyone boards buses to Black Estate	
12.30 pm	Arrive at Black Estate for lunch - Wine must be purchased now if your guests want!	Lunch provided including tea and coffee – make yourself known if you have noted dietary requirements. Purchase of your own Alcoholic beverages Wine purchase available at this time only

2.45 pm	Buses depart for the second tour	We now swap and do the other tour – so make sure on the right bus
3.15 pm	Tours commence	Please note Bathrooms at each location and easy walk from the bus to the tour. Tours are each 1.5 hours.
4.45 pm	Everyone Boards Buses	Back to Christchurch and Creyke Road University bus stop
6.00 pm	Disembark	Organise your own transport back to your accommodation and the evening is your own.

Symposium programme

Tuesday 27 August

1700-1900	Symposium registration
1900-1910 E5 Engineering	**Welcome address** Ren Dobson, Chair AUC2019
1910-2000 E5 Engineering	**Session 1: Svedberg Lecture** Chair Ren Dobson

Svedberg Lecture

Professor Borries Demeler

Canada 150 Research Chair for Biophysics

I will review some of the key contributions to the field of analytical ultracentrifugation over the past 25 years from our group, such as hydrodynamic models, the UltraScan-LIMS collaboration and supercomputing platforms for the use of high-resolution fitting of sedimentation velocity experiments. Particular focus will be given to the great potential multi-wavelength AUC analysis, which is now well represented in the UltraScan software suite. I will conclude with a preview of our latest development, UltraScan-GMP, a fully automated data acquisition system for the Optima AUC.

Wednesday 28 August - E5 Engineering Core.

0930-1055	**Session 2: Complementary methods: Scattering techniques (room E5)** **Chairs: Karen Fleming & Grant Pearce**
0930-0950	**Jill Trewhella -** *Reliable biomolecular structural modelling with small-angle scattering*
0950-1005	**Christine Ebel** - *Development of a SANS strategy for the study of membrane proteins: application to a prokaryotic NADPH oxidase homolog*
1005-1020	**Ivo Nischang** - *Hydrodynamic and light scattering study of very disperse and non-ideal macromolecule populations - Example of sodium carboxymethyl celluloses*
1020-1035	**Elizabeth Rodriguez** - *Higher order structure and conformational changes in biotherapeutics*
1035-1055	**Olwyn Byron** - *The spins: bacterial aldehyde-alcohol dehydrogenase forms spiral complexes critical for activity*
1055-1125	Morning Break
1125-1315	**Session 3: Method, hardware & software development (room E5)** **Sponsored by Beckman Coulter** **Chairs: Borries Demeler & Akash Bhattacharya**
1125-1145	**Tom Laue** - *High concentration IgG solutions*
1145-1205	**Walter Stafford** - *SEDANAL v7.1; Non-ideality at high concentrations, new features, and improvements*
1205-1225	**John Correia** - *Analysis of nonideality: Insights into fitting of high concentration therapeutic proteins in human serum*
1225-1240	**John Philo** - *Dealing with strong non-ideality in self-association: lessons learned from teduglutide*
1240-1255	**Wenqi Li** - *Analytical ultracentrifugation reveals poly acidic or basic amino acids sequence alters protein's hydrodynamic properties*
1255-1315	**Karen Fleming** - *HullRad: a fast, new tool for prediction of hydrodynamic parameters from structure*
1315-1405	Lunch
1325-1400	**Izon Workshop (room E6)**
1405-1530	**Session 4: Nanoparticles (room E5)** **Chairs: Helmut Cölfen & Johannes Walter**
1405-1425	**Helmut Cölfen** - *Emulsification of ultra-flexible microemulsions (UFME) triggered by soft Centrifugation*
1425-1440	**Guillermo González-Rubio** - *Analytical ultracentrifugation for understanding the role of cluster nature in the seed-mediated growth of gold nanocrystals*
1440-1500	**Johannes Walter** - *Accessing core-shell properties and functionalization of nanoparticles by means of analytical ultracentrifugation*
1500-1515	**Louis Weber** - *Analysis of Ti/NbCN nanoparticles extracted from microalloyed steels by SV-AUC*
1515-1530	**Xufeng Xu** - *Concentrated colloidal nanoparticles in analytical ultracentrifugation*
1530-1555	Afternoon Break (in the atrium)
1555-1730	**Session 5: Biological applications: Protein-ligand/nucleic acid interactions (room E5)** **Sponsored by Izon** **Chairs: Grant Pearce & Philipp Koldewey**
1555-1615	**Luitgard Nagel-Steger** - *Ligand interactions of amyloid-β protein studied by analytical ultracentrifugation in concert with microscale thermophoresis*
1615-1630	**Fareeda Barzak** - *Modelling APOBEC3-ssDNA bound complexes using small-angle X-ray scattering*
1630-1645	**Richard Kingston** - *Models for the site-specific treatment of cooperative ligand binding by proteins*
1645-1700	**Vlad Dinu** - *Sedimentation velocity studies of two salivary glycoproteins: possible structure-function effects of food flavour*
1700-1715	**Chris Horne** - *The repressor, depressor of sialic acid catabolism*
1715-1730	**Sarah Atkinson** - *Biophysical characterisation of novel Flavivirus antivirals that target host nuclear transport*
1730-1900	Mixer & Poster session Atrium?
1845-2300	Symposium dinner – Riccarton House (buses from UC leave at 1845)

Thursday 28 August - E5 Engineering Core.

0900-1020	**Session 6: Polymers (room E5)** **Chairs: Trushar Patel & Walter Stafford**
0900-0925s	**Simon Wawra** - *Determination of two-dimensional size distributions of plasmonic nanoparticles via the optical back coupling method*
0925-0950	**Igor Perevyazko** - *Hydrodynamic analysis of co-polymers with a gradient structure: solution properties, molar mass and conformation*
0950-1005	**Xiaodong Ye** - *Characterization of linear and cyclic polystyrene sulfonates using analytical ultracentrifugation*
1005-1020	**Jennifer Wakefield** - *AUC analysis of chitosan and aminocellulose for use in archaeological wood conservation*
1020-1050	Morning Break (in the atrium)
1050-1245	**Session 7: Biological Applications: Protein complexes (room E5)** **Chairs: Tatiana Soares da Costa & Sarah Atkinson**
1050-1110	**Davide Comoletti** - *A proteomic screen of neuronal cell surface molecules reveals IgLONs as structurally conserved interaction modules at the synapse*
1110-1130	**Trushar Patel** - *Towards studying nano-scale structures of Flaviviral non-coding RNAs-host protein complexes.*
1130-1150	**Ute Curth** - *E. coli primase and DNA polymerase III holoenzyme are able to bind concurrently to a primed template during DNA replication*
1150-1210	**Yee Mok** - *Tracking polyglutamine and polyalanine aggregates in cells using fluorescence-detected analytical ultracentrifugation*
1210-1230	**Grant Pearce** - *How does Rubisco activase activate Rubisco?*
1230-1245	**Akash Bhattacharya** - *What happened to the hiv capsid? A tale of armored viruses, goldilocks zones, molecular staples and traitorous co-factors*
1245-1400	Lunch (in the atrium)
1300-1355	**Beckman Coulter workshop (room E6)**
1400-1525	**Session 8: Novel uses of AUC (room E5)** **Chairs: Olwyn Byron & Jill Trewhella**
1400-1420	**Alexander Bepperling** - *High concentration AUC analysis of biopharmaceuticals*
1420-1435	**Tess Malcolm** - *Metal-dependent dynamic equilibrium: A mechanism for regulation of the Plasmodium M17 aminopeptidases?*
1435-1450	**Arthur Gadon** - *Characterisation of high molecular weight hop proanthocyanidins using analytical ultracentrifugation*
1450-1505	**Grzegorz Piszczek** - *Single molecule versus bulk detection: Interferometric light scattering (iSCAMS) and AUC applied to study protein-protein interactions*
1505-1525	**Chad Brautigam** - *Modern approaches to the detection of ligand-induced protein conformational changes using AUC*
1525-1545	Afternoon Break (in the atrium)
1545-1800	**Session 9: Biological Applications: Protein structures (room E5)** **Chairs: Ren Dobson & Luitgard Nagel-Steger**
1545-1605	**David Goldstone** - *Structural and biophysical characterisation of the master regulator TRIM28*
1605-1625	**Beata Wielgus-Kutrowska** - *Adenylosuccinate synthetase from Helicobacter pylori: biochemical and biophysical characteristics including comparison of C- and N-terminal His-tag variants*
1625-1645	**Stephen Perkins** - *The solution structure of the human IgG2 subclass is distinct from those for human IgG1 and IgG4, providing an explanation for their discrete functions*
1645-1700	**Valentina Spiteri** - *Unravelling the solution structures and stabilities of therapeutic antibodies with and without glycans*
1700-1720	**Mike Griffin -** *Understanding the mechanism of a lead interleukin-11 signalling inhibitor*
1720-1800	**Closing comments and business meeting**

Workshop abstracts

1-2) Introduction into AUC Workshop

Introduction into Hydrodynamics and Solution Studies by Analytical Ultracentrifugation

Presenter: Tom Laue (University of New Hampshire, USA)

Description:

My introductory talk will cover the following:

Session 1

1. Simple AUC theory
    a. Contributors to S- shape, size, etc.
    b. Dilute solutions
2. AUC practices
    a. Components
    b. Simple systems
    c. Complex systems

Session 2

3. Optical systems
    a. Kinds
    b. How they work
    c. How to select
4. Complex and concentrated solutions
    a. How to deal with them

3) Fluorescence Spectroscopy: a complement to AUC

Presenter: Sandy Ross and Harmen Steele (University of Montana, USA)

Description

This workshop is an introduction to fluorescence methods used in the life sciences and nanotechnology to study questions about dynamics and hydrodynamics. We will review the basics of steady-state and time-resolved fluorescence measurements. Topics to be covered include: Förster resonance energy transfer (FRET), which is often used to study conformational change and distances between 1-10 nm; fluorescence anisotropy (steady-state and time-resolved), which can be used to study nanosecond-timescale dynamics of membranes and macromolecules, such as DNA and proteins; and fluorescence correlation spectroscopy (FCS), which can be used to determine translational diffusion coefficients of biological molecules and their assemblies, including membranes.

4) Workshop on AUC of Membrane Proteins

Presenter: Karen Fleming (Johns Hopkins University, USA) and Christine Ebel (University Grenoble Alpes, France)

Description

Integral membrane proteins (MPs) are physiologically embedded in a lipid bilayer, which provides a hydrophobic environment compatible with their nonpolar, transmembrane surfaces. Both structural and solution studies of MPs often involve their extraction, solubilization, purification, and characterization in a wide variety of detergent micelles. In this workshop, we will describe the AUC characterization and analysis of detergent solubilized membrane proteins. Parameters that can be evaluated include MP homogeneity, protein molar mass, bound detergent, and protein-protein association constants.

The AUC challenge of solubilized membrane protein samples is principally that detergent-solubilized membrane proteins represent a multicomponent system, in which different components (protein, detergent, lipid, water?) interact to form the different types of sedimenting particles (species). The samples are therefore necessarily polydisperse, principally because of the presence of free detergent micelles in solution. Depending on the specific conditions, this complexity can be disentangled by taking advantage of several key distinctions between protein and detergent properties. For example, detergents and proteins have distinct optical properties (different extinction coefficients and increments of refractive index), and different buoyant properties (partial specific volumes). Moreover, membrane protein-protein interactions (as protein stability) can significantly depend on the type and concentration of detergent. In addition to a presentation of the theoretical considerations and mathematical formalism, we will discuss practical implementation and analysis of both sedimentation velocity and sedimentation equilibrium experiments and analysis of membrane protein/detergent solutions. Topics to be covered include the following:

Sedimentation Velocity

- How to determine the parameters needed for data analysis (partial specific volumes and increment of refractive indexes)
- Strategies for the design of the cells and experiments
- How to analyze the data using sedfit/sedphat/gussi programs
- How to evaluate size distribution and non-interacting species, sample homogeneity, protein association state, amounts of bound detergent, and the limits of these analysis.

Sedimentation Equilibrium

- How to determine parameters needed for data analysis (partial specific volumes)
- Strategies for experimental design, including how to density match and what to do if density matching using water is not feasible
- How to globally analyze the data using WinNonlin
- How to evaluate molecular weight distributions for reversible equilibrium

5) US-SOMO Hydrodynamic Modelling

UltraScan Solution Modeler Hydrodynamic Modelling Workshop

Presenters: Emre Brookes (University of Montana, USA)

Description

UltraScan Solution Modeler (US-SOMO) processes atomic and lower-resolution bead model representations of biological and other macromolecules to compute various hydrodynamic parameters, such as the sedimentation and diffusion coefficients, relaxation times and intrinsic viscosity. This allows the researcher to validate structural models against experimental data. The tools available in US-SOMO have been shown to provide the best-known computations of hydrodynamic parameters from experiment [1]. The US-SOMO hydrodynamics workshop will consist of three sessions. In the first session, attendees will be given an introduction to computational hydrodynamics and techniques. In the second session, users will utilize US-SOMO to compute hydrodynamics parameters from structures and to use automated batch processing to compute over multiple structures. In the third session, additional examples will be presented. If users wish to bring their own structures, we will assist you at this time to process them with US-SOMO.

It is suggested that attendees attend, at minimum, any prior sessions, as earlier material will not be covered again during the 3 session course. Additionally, registered attendees will receive detailed software installation instructions in the week prior to the workshop. We will provide support to resolve any issues during this time.

[1] Rocco M, Byron O., 2015, "Hydrodynamic Modeling and Its Application in AUC.", Methods Enzymol. 2015;562:81-108. doi: 10.1016/bs.mie.2015.04.010.

6) US-SOMO Small Angle Scattering

Presenters: Emre Brookes (University of Montana, USA)

Description

The UltraScan Solution Modeler (US-SOMO) has, in addition to hydrodynamic parameter calculation from structure, native and wrapped tools for computation of solution small angle scattering data from biomacromolecules (BioSAS), processing of BioSAS data and comparison of simulated data against experimental data. US-SOMO also contains powerful tools for the analysis of size exclusion coupled small angle X-ray scattering (SEC-SAXS) data. In this three-session workshop, we will begin with an introduction to BioSAS. This will begin with an overview of the basics of BioSAS and its importance to the researcher. Next, during the first session, we will introduce the attendees to the available BioSAS tools within US-SOMO. In the second sessions, the attendees will work through hands-on exercises using US-SOMO with a focus on the SEC-SAXS tools. In the final session, we will continue with additional example data sets and offer to look at any user supplied data.

It is suggested that attendees attend at least any prior sessions of this three-session workshop, as earlier material will not be covered again. Additionally, registered attendees will receive instructions to install the software in the week prior to the workshop. We will provide support to resolve any issues during this time.

7) Introduction into SASSIE2

SASSIE: Software to Model AUC & SAS Data Using Atomistic Ensembles from Molecular Simulations.

Presenters: Emre Brookes (University of Montana, USA) and Stephen Perkins, Division of Biosciences, University College London, UK

Description

Molecular simulation is an important technique to analyze and interpret molecular phenomena across many disciplines. AUC & Small-angle scattering (SAS) utilizing either light or neutron sources are valuable methods to characterize shape, interactions, and properties of many soft-matter systems. Modeling of AUC & SAS data is often done using analytical functions and/or dummy-ball (DB) models. While these methods are simple, they have proven to be quite robust and have allowed for a tremendous expansion of AUC & SAS studies to a wide variety of systems. Atomistic modeling can be used to interpret AUC & SAS data and inherently provides structural and atomic interactions that are unavailable using analytical or DB models. In addition, atomistic models can allow the use of experimental and computational constraints on the experimental data. The goal of this introductory course is to use modern simulation methods and software tools to predict and analyze AUC & small-angle scattering data of biological systems. The bulk of the course will introduce SASSIE, a software framework designed to facilitate the use of atomistic modeling to interpret AUC & scattering data. The course will involve a mixture of lectures and examples with student lessons. Examples will involve various protein and DNA molecules, as well as their complexes. The emphasis will be on structure building, ensemble molecular simulation, calculation of AUC & scattering profiles, and comparison to experimental data.

Session 1: Introduction to molecular modeling using force-fields.
Session 2: SASSIE.
Session 3: Calculating AUC and SAS Profiles from Simulation.
Session 4: Advanced Topics

8) AUC of Nanoparticles

Workshop and Discussion about Challenges in the AUC of Nanoparticles

Presenter: Helmut Cölfen (University of Konstanz, Germany)

Description

Analytical Ultracentrifugation (AUC) was originally invented for the analysis of nanoparticles. It is still a great tool for their analysis. However, Nanoparticles pose special challenges to the researcher, which are typically not encountered in the analysis of proteins. These are:

- Polydispersity
- Hybrid character and therefore folded particle size and particle density distribution
- Stabilization by charge, which cannot be shielded by buffers
- A size which already leads to light scattering superimposing the signal by absorption
- Shape distributions
- Size dependent optical properties

The workshop will be in form of a presentation demonstrating solutions to these challenges as well as a discussion. These strategies can also be adapted to extremely polydisperse Biopolymers and complexes, which also have some of the above problems in common with nanoparticles. Users are encouraged to bring their own examples with them for discussion.

9) Introduction to UltraScan and UltraScan Data Analysis

Presenter: Borries Demeler (University of Texas, USA)

Description

In this session, I will present an easy introduction into UltraScan, discuss the basic concepts underlying data analysis with UltraScan and help participants become familiar using a database backend for data management. I will go over the LIMS system and remote supercomputer analysis, and discuss the layout of UltraScan modules and data files, and review the data workflow when performing data analysis. Participants will learn how to commit data, perform the basic workflows for data processing if sedimentation velocity analysis, and use the supercomputer backend, utilize the reporting system, and manipulate data both locally and in the database. Participants will learn how to upload and edit absorbance, intensity, fluorescence and interference data, and how to retrieve data from the new Optima AUC. We will review the data manager and other utilities to help you access your data remotely from different computers and resume analysis from different sites.

Workshop participants must bring a wifi capable laptop with the latest version of UltraScan installed on it, and registered a LIMS account in the UltraScan Demo LIMS instance.

10) AUC Problem Solving with UltraScan, Introduction to UltraScan and UltraScan Data Analysis

Description

During this session, I will focus on advanced topics in experimental design, data analysis and result interpretation. We will review the information obtained from various analysis methods and compare and contrast what can be learned from each method (and what cannot be learned!). We will discuss the 2-dimensional spectrum analysis, custom grid method, genetic algorithms, the parametrically constrained spectrum analysis, the Monte Carlo analysis, the van Holde - Weischet analysis and discrete model genetic algorithms for fitting interacting systems and custom-built models.

I will also review strategies for maximizing experimental information by judiciously designing your experiments, and discuss caveats, tips and tricks that are often overlooked. This will be done by example using sample data from systems that illustrate the following concepts:

- Reversible self-association
- Non-interacting Systems
- Particle Sizing
- Aggregation analysis
- Global fitting of non-interacting systems
- Multi-speed analysis
- Global fitting of multi-speed systems

Another part of this workshop will look at visualizations for finite element models using 2-dimensional and 3-dimensional plotting procedures, and if time permits, I'll present advanced topics in multi-wavelength analysis and cover how publication-quality custom graphs can be generated with UltraScan.

Please note:

1. The morning sessions are prerequisite for participation in the afternoon sessions unless prior arrangements have been made with the workshop presenter.
2. Workshop participants must bring a wifi capable laptop with the latest version of UltraScan installed on it, and registered a LIMS account in the UltraScan Demo LIMS instance. To sign up, please visit https://uslims3.fz-juelich.de/uslims3_US3_DEMO/newaccount.php

11) SEDANAL Workshop

Introduction into SEDANAL

Presenter: Walter Stafford, Harvard Medical School and Jack Correia, University of Mississippi

Session Listing:

- SEDANAL - Introduction and Sedimentation Velocity of Noninteracting Systems (Sunday, 9:00-13:30)
- SEDANAL - continued. Sedimentation Velocity of Interacting Systems (Sunday, 14:30-19:00)
- SEDANAL - continued. Sedimentation Velocity of Non-ideal Systems (Monday, 9:00-11:00)
- SEDANAL - continued. Sedimentation Equilibrium of Non-interacting and Interacting Systems (Monday, 11:30-13:30)
- SEDANAL - continued. Advanced Topics: Multiwavelength Analysis (Monday, 14:30-19:00)

Attendees who sign up for the SEDANAL Workshop are encouraged to attend both of the Monday sessions since the subsequent sessions will move on quite rapidly without revisiting the basic use of the software. For example, the introduction will cover the setup, loading and initial handling of the data, and that will not be repeated in detail in the later sessions. The last two sessions could be skipped depending on specific interest in those topics.

12) SEDNTERP

Presenter: John Philo (Alliance Protein Laboratories)

Description:

Introduction to SEDNTERP and its use for calculating solvent density and viscosity, partial specific volumes, and actual experimental hydrodynamic parameters for your favorite molecules (*e.g.* for comparisons with calculated values from bead modeling). This workshop would also provide background for similar calculations that have been implemented (at least to some extent) in other software packages such as ULTRASCAN or SEDFIT. This is a lecture/demo rather than a hands-on software (attendees will not need to install any particular version of SEDNTERP).

13) Time-derivative Analysis with the Program DCDT+

Introduction to dc/dt analysis and hands-on software workshop

Presenter: John Philo (Alliance Protein Laboratories)

Description

Introduction to sedimentation velocity analysis using the time-derivative method and the user-friendly program DCDT+. How to use g(s*) distributions as a model-independent way to test for reversible self-association and to determine the molar mass of the main component despite the presence of minor components.

14) Getting Confidence Limits for the Properties of Each Peak in Your c(s) Distribution Using the Program SVEDBERG

A hands-on software workshop

Presenter: John Philo (Alliance Protein Laboratories)

Prerequisites: at least some familiarity with c(s) analysis in SEDFIT (or similar size-distribution methods in ULTRASCAN)

Description

Although sedimentation coefficient distributions are widely used to identify what species (peaks) are present in a sample, it is usually not straightforward to determine the confidence limits for the properties of those species. In particular, SEDFIT provides no information about the precision of the peak fractions, which in many cases is the property of most interest. Furthermore, although SEDFIT can use Monte-Carlo approaches to try to assess a confidence region for each point (sedimentation coefficient) in the c(s) curve, that approach can be misleading because it doesn't explicitly deal with the fact that there is uncertainty in the position of each peak. A third important point is that Monte-Carlo approaches assume the noise in the raw data is randomly distributed, which is not ever really true for AUC data.

This workshop will show you how to use the user-friendly, public-domain program SVEDBERG to easily translate your c(s) distribution to a mixture model and obtain confidence limits for the peak fractions, sedimentation coefficient, and the molar mass for each peak using any of three different statistical approaches (including the bootstrap method, which makes no assumptions about the noise in the data being random).

The workshop will also describe how we can use SVEDBERG to sequentially release the built-in constraint of the c(s) method that every species has the same hydrodynamic shape (f/f0 ratio), and thereby learn how much information about the molar mass (or shape) of each species is really present in the raw data.

15) Hydrodynamic analysis of synthetic and bio-macromolecules

Complementary analytical techniques: Analytical ultracentrifugation, intrinsic viscosity, and light scattering

Presenters: Ivo Nischang (Friedrich Schiller University Jena, Germany) and Igor Perevyazko (St. Petersburg State University, Russia)

Description

1. Conformational properties and solution behavior of macromolecules – basics: Molar mass, hydrodynamic size, end-to-end distance of polymer chains, conformational state (hard sphere, random coil, rigid rod), equilibrium rigidity, and diameter of polymer chains, dilute and semi-dilute polymer solutions, solvent quality
2. Complementary / orthogonal hydrodynamic methods:
    a. Viscometry of macromolecules: Intrinsic viscosity and absolute (dynamic) viscosity, degree of dilution, viscometric parameters, charged and neutral macromolecules
    b. Sedimentation velocity analysis of macromolecules: Heterogeneity of samples, LAMM equation modelling, concentration dependence of sedimentation coefficient and frictional ratio, Gralen coefficient, molar mass estimations, charged and neutral macromolecules
    c. Translational diffusion measurements of macromolecules: Measurements of diffusion by synthetic boundaries, diffusion coefficients by dynamic light scattering (DLS), concentration dependences of diffusion coefficients, hydrodynamic radii / diameters (Stokes-Einstein)
3. Hydrodynamic invariants: Consistency of the experimental data: Hydrodynamic invariant, A0, and sedimentation parameter, βs
4. Complementary physical methods - separations and light scattering Size exclusion chromatography (SEC), asymmetrical flow field-flow fractionation (AF4), multi-angle laser light scattering (MALLS) – molar masses, radii of gyration and their distributions
5. Absolute molar mass and hydrodynamic size in solution
6. Conformation and conformational characteristics: Macromolecular shape and conformation in solution, Kuhn-Mark- Houwink-Sakurada scaling relationships, evaluation of equilibrium rigidity and diameter of polymer chains
7. Strategy to study solution properties of synthetic and bio- macromolecular samples of unknown properties

16) GUSSI for Illustration of AUC and Other Biophysical Data.

Presenter: Chad Brautigam (The University of Texas Southwestern Medical Center, USA)

Description

An overview of GUSSI and its features.

In all workshops, the idea would be to give lectures on the theory behind the subject matter, and then guide the students through analytic exercises using a distributed set of data and software.

17) Membrane proteins and conformational changes

Workshop on SEDFIT and GUSSI

Presenter: Chad Brautigam (The University of Texas Southwestern Medical Center, USA)

Description

Session 5: Title: SV to Determine the Oligomeric States of Membrane Proteins & Glycoproteins.

Synopsis: Using SEDFIT and GUSSI to determine oligomeric states for proteins with associated detergents or carbohydrates.

Session 6: Title: Determining Conformational Changes in Proteins Using SV.

Synopsis: Using conventional SV and difference SV to estimate the magnitude of a ligand-induced conformational change in a protein.

In all workshops, the idea would be to give lectures on the theory behind the subject matter, and then guide the students through analytic exercises using a distributed set of data and software.

18) Multisignal SV and Analyzing SE Data

Presenter: Chad Brautigam (The University of Texas Southwestern Medical Center, USA)

Description

Session 7: Title: Multisignal SV.

Synopsis: Using multisignal SV to determine the stoichiometry of co-sedimenting macromolecular complexes.

Session 8: Title: Sorting and Analyzing SE Data.

Synopsis: Using GUSSI to sort SE data and using these sorted files in downstream analysis in SDPHAT.

In all workshops, the idea would be to give lectures on the theory behind the subject matter, and then guide the students through analytic exercises using a distributed set of data and software.

19) Complementary Techniques: ITC and MST

Presenter: Chad Brautigam (The University of Texas Southwestern Medical Center, USA)

Description

Sessions 9: Title: ITC Data Analysis.

Synopsis: Using NITPIC, SEDPHAT, and GUSSI to integrate, analyze, and illustrate ITC data.

Session 10: Title: MST Data Acquisition and Analysis.

Synopsis: Advanced approaches to the analysis of MST data using PALMIST and illustrating MST data in GUSSI.

In all workshops, the idea would be to give lectures on the theory behind the subject matter, and then guide the students through analytic exercises using a distributed set of data and software.

20) HDR-Multifit

HDR-MULTIFIT – Analysis of Turbidity Data and Determination of Particle Size Distributions

Presenter: Johannes Walter, FAU, Germany

Description

Analytical Ultracentrifugation is perfectly suited for the analysis of particle size distributions (PSDs). However, the characterization of polydisperse PSDs is making high demands to any characterization technique. For sedimentation analysis, it has to be taken into account that the sedimentation rate scales with the particle size squared. To tackle such challenging systems, MWL gravitational sweep (GS) experiments at a fixed radial position were developed which are based on a continuously increasing rotor speed. GS experiments are of particular importance when studying polydisperse PSDs due the much larger dynamic range compared to traditional sedimentation velocity (SV) experiments.

HDR-MULTIFIT can be used to analyze GS data and provides the ability to determine the optical properties of individual components in polydisperse mixtures and to relate this data to the hydrodynamic properties. For spherical NPs having well-defined refractive indices, high dynamic range (HDR) particle size analysis is possible. The analysis benefits from the direct fractionation of different particle sizes in the measurement cell during sedimentation, while the optimum signal to noise ratio for the whole PSD is achieved by automatically tuning the wavelength used for data evaluation.

Besides GS experiments, it is demonstrated that HDR-MULTIFIT can also be used for the post-processing of sedimentation coefficient distributions from sedimentation velocity experiments (derived by e.g., SEDFIT, SEDANAL, Ultrascan, DCDT+). Concentration coupling and the simultaneous determination of size and density are further capabilities offered by HDR-MULTIFIT.

The workshop will tackle the following topics:

- Determination of particle sizes and their distributions
- The influence of scattering and the role of Mie’s theory
- Correction of size distributions and their weighting
- Multiwavelength analysis of GS experiments
- Concentration coupling experiments for polydisperse distributions
- Simultaneous analysis of size, density and refractive index increment using density variation

Most importantly, this workshop will include a hands-on training on HDR-MULTIFIT.

Symposium abstracts

Reliable biomolecular structural modelling with small-angle scattering

Jill Trewhella[1]

[1] *The University of Sydney, Australia*

This presentation will focus on the recommended reporting framework for biomolecular small-angle scattering (SAS) and its rationale, and provide a progress report on a follow-on project that is underway to develop a consensus standard benchmark data set for evaluating different approaches to predicting scattering profiles from atomic coordinates. This latter effort has to date engaged more than three dozen scattering experts from around the world, with a set of scattering measurements being made on a standard set of 5 proteins at 14 facilities across Asia, North America and Europe.

X-ray or neutron SAS from biomolecules in solution can provide (1) unique structural insights, (2) information on the range of potential conformations present, (3) and powerful restraints for integrative/hybrid structural modelling (IHM). The rotational and ensemble averaging of the signal presents multiple challenges to the reliable utilisation of SAS data: (1) demonstrating SAS profile represents the species of interest, (2) reliably evaluating model fits, and (3) avoiding over-interpretation and over-fitting of data. A community driven project has been underway for more than a decade now to define the information that should be made available when publishing biomolecular SAS experiments. These guidelines (Trewhella et al. (2017) *Acta D73*, 710) provide a reporting framework so that "readers can independently assess the quality of the data and the basis for any interpretations presented" concerning the results of bimolecular SAS experiments, including the generation or testing of 3D models. The validation task forces for SAS (Trewhella et al. (2013) *Structure 21*, 875) and for IHM (Sali et al. (2015) *Structure 23*, 1156) of the world-wide Protein Data Bank are additionally addressing the archiving and validation of IHM models that depend upon computational methods and disparate data from multiple techniques, including SAS (Burley et al. (2017) *Structure 25, 1317*). The development of publication guidelines and the standard benchmark data are essential steps to ensuring confidence in the results of biomolecular SAS so that it can contribute optimally to the growing field of IHM structure determination.

Development of a SANS strategy for the study of membrane proteins: application to a prokaryotic NADPH oxidase homolog

Vermot Annelise[1], Petit-Hartlein Isabelle[1], Breyton Cécile[1], Le Roy Aline[1], Deniaud Corinne[1], Thépaut Michel[1], Hartlein Michaël[2], Smith Susan[3], Ebel Christine[1], Martel Anne[2], Fieschi Franck[1]

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Small angle neutron scattering (SANS) provides a method to characterize transmembrane systems without necessity for a crystallization step; although of low resolution, SANS furnishes valuable data regarding organization of macromolecular membrane complexes, conformational state modifications from one state to another, etc. Membrane protein solubilization implies the use of amphipathic detergents that form protective belts around hydrophobic patches to maintain the protein's structural integrity outside of the lipid environment. Consequently, the resulting SANS signal includes both protein and detergent belt signatures leading to a global outer shell larger than the envelope expected for the protein alone; masking the detergent signal represents an important issue. Moreover, specific detergent characteristics can disrupt the proper folding of the membrane protein, greatly impacting the structural parameters of the SANS envelope and thus necessitating significant effort in the preparation of an optimized sample for SANS. Thus, a strategy to overcome these specific issues was tested using the LMNG detergent, recently characterized as a strongly stabilizing detergent. As a model protein we used SpNox, a prokaryotic homolog of NADPH oxidase originally identified in phagocytic cells where it produces ROS. Detergent screening confirmed the improved thermostability of SpNox and limited aggregation when solubilized in LMNG. Through contrast variation experiments, we were able cancel the LMNG contribution to the SANS signal, and we determined the percentage of D2O corresponding to the buffer matching point. Then, to improve protein contrast, we produced deuterated SpNOX and subsequently solubilized and purified it in LMNG. Finally, since SANS studies are very sensitive to the presence of aggregates or oligomers in the sample, the development of a new in-situ size exclusion chromatography (SEC) system on a SANS instrument enabled data collection on line from an aggregation-free homogeneous sample. This strategy led to the determination of a low resolution envelope of SpNox confirming the monomeric state of the protein in solution. Parallel to the structural study, a homology model of SpNox was developed; the model was successfully docked in the SANS envelope. The general strategy illustrated with the SpNox protein may be applied to many other membrane proteins in the future.

Hydrodynamic and light scattering study of very disperse and non-ideal macromolecule populations - Example of sodium carboxymethyl celluloses

Ivo Nischang[1]

[1]*Laboratory of Organic and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena; Jena Center for Soft Matter (JCSM), Friedrich Schiller University Jena, Philosophenweg 7;*

Cellulose is one of the most abundant natural macromolecule and commercially available in a variety of forms. One of the most significant markets concerns carboxymethyl cellulose (CMC) because of its widespread application as a biomaterial and in industrial processes. Therefore, CMCs are studied for a long time by a selection of techniques. Associated to applications in medicine, pharmacy, biology, and particularly as components of biomaterials, its fundamental properties on a molecular scale are of high importance. However, CMCs are known for their dispersity, which may impose problems for a more fundamental molecular characterization.

We approach this characterization challenge by two seminal macromolecular characterization techniques in solution. These techniques comprise molecular hydrodynamic analysis via viscometry and sedimentation analysis in analytical ultracentrifugation (AUC) as well as size exclusion chromatography (SEC) coupled to multi-angle laser light scattering (MALLS). We do this over a wide range of molar masses and desired degrees of dilution.

We start our approach with the determination of intrinsic viscosities followed by the calculation of the degree of dilution. In the following, we perform sedimentation velocity experiments via AUC. Here, we could verify the range of concentrations suitable for sedimentation velocity experiments to perform the extrapolation to infinite dilution, aiming at reconciliation of the molecular hydrodynamic properties. Consequently, molar masses are determined by SEC-MALLS.

All of the hydrodynamic parameters of a particular CMC sample - intrinsic viscosity, intrinsic sedimentation coefficient, molar masses / diffusion coefficients - are subsequently shown to interrelate via a hydrodynamic invariant concept that demonstrates the quantitative nature of our approach. This quantitative nature is further underpinned by physical interrelation of scaling relationships. Our approach provides an opportunity for a comprehensive characterization of biomaterials in solution even at very high degrees of dispersity. This includes valuable information to establish quantitative structure-property relationships of biomaterials.

Higher Order structure and conformational changes in biotherapeutics

Elizabeth Rodriguez[1]

[1] *UCB Bell Tech, UK*

The higher order structure (HOS) of protein biopharmaceuticals is critical for biological and pharmacological function. Changes in higher order structure can impact efficacy, stability, safety and immunogenicity. The technology and techniques available for HOS characterisation have advanced in recent years complimenting or replacing traditional methods such circular dichroism (CD) and Fourier transform-infrared spectroscopy (FTIR) . In this study we applied small-angle X-ray scattering (SAXS) and a range of other non-routine techniques to elucidate the structure of an antibody and demonstrate how process changes can result in structural changes and impact long term stability. We show that a gain in conformational stability in the HOS was likely linked to the increase in colloidal stability. This study highlights the need to use non-routine techniques to detect subtle conformational changes to enable understanding and comprehensive characterisation of biopharmaceuticals during development, manufacture and storage.

The spins: bacterial aldehyde-alcohol dehydrogenase forms spiral complexes critical for activity

Gijeong Kim[1], Liyana Binti Azmi[2], Seongmin Jang[1], Taeyang Jung[3], Hans Hebert[4], Andrew Roe[2], Olwyn Byron[5], Ji-Joon Song[1]

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Escherichia coli O157:H7 (EHEC) is a pathogenic strain of *E. coli* and the causative agent of bloody diarrhoea, severe colitis and haemolytic uraemic syndrome. These conditions arise owing to the production of Shiga toxin, which is released upon exposure to wrongly prescribed antibiotic treatment. In fact, supportive care is the only current treatment for EHEC. This unmet need, together with the rise in antimicrobial resistant (AMR) bacteria, is driving an effort towards the discovery antibiotic alternatives to treat EHEC.

As part of this effort, high-throughput screening of salicylidine acylhydrazide (SA) compounds was previously shown [1] to suppress the expression of the type three secretion system (T3SS) and disable EHEC motility. To understand the mode of action of these compounds, their cellular targets were identified and characterised. Only the deletion of *adhE* resulted in T3SS down-regulation and overexpression of non-functional flagella, replicating the phenotype observed in the presence of SA compounds. However, the molecular basis underlying this phenotype is unknown.

AdhE (aldehyde-alcohol dehydrogenase) is a key enzyme in bacterial fermentation, converting acetyl-CoA to ethanol, via two consecutive catalytic reactions. In order to be able to pursue structure-based design of compounds suitable as potential drugs, the high-resolution structure of AdhE was sought. However, AdhE is known to form heterogeneous spiral super-complexes (spirosomes) that preclude crystallisation of the monomeric protein. In this talk I will describe how we have used our favourite biophysical tools (AUC and SAXS) for the structural study of AdhE and its complexes, and report on a recent fortuitous, fruitful and ongoing collaboration with colleagues who have determined the high-resolution structure of AdhE within the spirosome and identified a single mutation that significantly alters spirosome assembly [2].

1. Wang, D., Zetterström, C. E., Gabrielsen, M., Beckham, K. S. H., Tree, J. J., Macdonald, S. E., Byron, O., Mitchell, T. J., Gally, D. L., Herzyk, P., Mahajan, A., Uvell, H., Burchmore, R., Smith, B. O., Elofsson, M., Roe. A. J. (2011) JBC, 286, 29922-31.
2. Kim, G., Azmi, L., Jang, S., Jung, T., Hebert, H., Roe, A., Byron, O., Song, J. (2019) (under review).

High concentration IgG solutions

Tom Laue[1], Danlin Yang[2], Jack Correia[3], Walter Stafford[4]

[1]*University of New Hampshire*
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[3]*University of Mississippi Medical Center*
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The nonideality of protein solutions comprises the sum of energies from repulsive and attractive interactions. A framework will be presented in which the energetics of the interaction process is broken into two parts, longer-ranged through-space colloidal interactions and close-approach desolvation and surface contact. This framework is applied to understand the underlying energetics leading to the concentration-dependent behavior of IgG solutions. Both the practical and biological consequences of these interactions will be discussed. Fluorescence-detected sedimentation uniquely supplies the information needed to characterize interactions in complex, concentrated solutions.

SEDANAL v7.1; Non-Ideality at high concentrations, new features, and improvements

W.F. Stafford[1], P.J. Sherwood[2]. and J.J. Correia[3]

[1]Harvard Medical School, Boston MA,
[2]Interactive Technology, Oakland CA,
[3]University of Mississippi Medical Center, Jackson, MS

Since 2015, when we made major changes to Sedanal to handle multiwavelength data, several more recent changes, additions and improvements have been made to handle non-ideality associated with high concentration regimes, such as fluorescent tracer sedimentation in serum. In addition, various parts of the computations have been parallelized (using OpenMP) to affect a several-fold increase in speed, plus numerous other small enhancements and tweaks. Non-ideality is handled using the "Ks Matrix" and the "BM1 Matrix" to account for all the direct and crossterm non-ideal interactions for non-interacting as well as interacting systems such that

$$\ln \gamma_i = \sum_j B'_{ij} C_j = \sum \mathbf{Bc}$$

where (e.g. for 3 species)

$$\mathbf{B} = \begin{vmatrix} B'_{11} & B'_{12} & B'_{13} \\ B'_{21} & B'_{22} & B'_{23} \\ B'_{31} & B'_{32} & B'_{33} \end{vmatrix} \text{ and } \mathbf{c} = \begin{vmatrix} c_1 \\ c_2 \\ c_3 \end{vmatrix}$$

where **B** is an I x J matrix of second virial coefficients and

$$\mathbf{f} = \mathbf{f_o}\,(1 + \mathbf{k_s c})$$

where (e.g. for 3 species)

$$\begin{vmatrix} f_1 \\ f_2 \\ f_3 \end{vmatrix} = \begin{vmatrix} f_{o,1} & 0 & 0 \\ 0 & f_{o,2} & 0 \\ 0 & 0 & f_{o,3} \end{vmatrix} \begin{vmatrix} 1 & k_{s,11} & k_{s,12} & k_{s,13} \\ 1 & k_{s,21} & k_{s,22} & k_{s,23} \\ 1 & k_{s,31} & k_{s,32} & k_{s,33} \end{vmatrix} \begin{vmatrix} 1 \\ c_1 \\ c_2 \\ c_3 \end{vmatrix}$$

where the $\mathbf{K_s}$ matrix is an I x J matrix of hydrodynamic concentration dependence coefficients, $K_{s,i,j}$ expressing both the direct and cross-terms. For example, for a 3 species system we have:

$$\begin{aligned} s_1 &= s_1^o / \left(1 + k_{1,1}c_1 + k_{1,2}c_2 + k_{1,3}c_3\right) \\ s_2 &= s_2^o / \left(1 + k_{2,1}c_1 + k_{2,2}c_2 + k_{2,3}c_3\right) \\ s_3 &= s_3^o / \left(1 + k_{3,1}c_1 + k_{3,2}c_2 + k_{3,3}c_3\right) \end{aligned}$$

$$\begin{aligned} D_1 &= D_1^0 \frac{\left(1 + B'_{1,1}c_1 + B'_{1,2}c_2 + B'_{1,3}c_3\right)}{\left(1 + k_{1,1}c_1 + k_{1,2}c_2 + k_{1,3}c_3\right)} \\ D_2 &= D_2^0 \frac{\left(1 + B'_{2,1}c_1 + B'_{2,2}c_2 + B'_{2,3}c_3\right)}{\left(1 + k_{2,1}c_1 + k_{2,2}c_2 + k_{2,3}c_3\right)} \\ D_3 &= D_3^0 \frac{\left(1 + B'_{3,1}c_1 + B'_{3,2}c_2 + B'_{3,3}c_3\right)}{\left(1 + k_{3,1}c_1 + k_{3,2}c_2 + k_{3,3}c_3\right)} \end{aligned}$$

In addition to incorporating non-ideality matrices into Sedanal, Wide Distribution Analysis (WDA) has been improved using better numerical methods. WDA uses all scans acquired and computes s*g(s*) vs log(s*) and can utilize data taken at either a single speed or a series of increasing speeds to accommodate a wide range of s values in a single experiment. Because WDA is essentially instantaneous, it has become the first method of analysis after acquiring the data. SEDANAL allows for compressibility ($\rho = \rho(r)$) and pressure dependence of viscosity ($\eta = \eta(r)$), as well as dynamic density $\rho = \rho(r,t)$. Multicomponent synthetic boundary and band sedimentation can also be analyzed by using an initial scan. Another program, developed by David Hayes, called SEDVIEW, uses similar algorithms as WDA and produces similar plots of s*g(s*) vs log(s*) and can process the scans in real time as they are acquired allowing one to follow the run as it evolves.

Analysis of Nonideality: Insights into Fitting of High Concentration Therapeutic Proteins in Human Serum

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The Aviv fluorescence detection system (Aviv-FDS) has allowed the performance of sedimentation velocity experiments on therapeutic antibodies in highly concentrated environments like serum and formulation buffers. Methods have been implemented in the software package SEDANAL for the analysis of nonideal, weakly associating AUC data acquired on therapeutic antibodies and proteins using absorbance or FDS optics (Wright, et al, Eur. Biophys. J. 47, 709, 2018; Wright, et al, Anal. Biochem. 550, 72, 2018). This involves determining both hydrodynamic nonideality Ks and thermodynamic nonideality BM1 where concentration dependence is expressed as s = s0/(1 + Ksc) and D = D0(1 + 2BM1c)/(1 + Ksc). To gain insight into the determination of these phenomenological parameters, we have performed simulations with SEDANAL of mAb as a function of Ks (0 to 100 ml/gm) and BM1 (0 to 100 ml/gm). This provides a visual understanding of the impact of Ks and BM1 on the shape of high concentration sedimentation boundaries and the challenge of their unique determination by finite element methods. The direct boundary global fitting of highly nonideal systems in SEDANAL is extremely difficult because high values of BM1c cause numerical instability at the base of the cell. To overcome this, we have implemented limitations on D/Do (Todd and Haschemeyer, PNAS 78, 6739, 1981; eta = (1-D/Do) in their nomenclature). In addition, mAbs all appear to undergo weak self- and hetero-association (Yang, et al., Prot. Sci. 27, 1348, 2018) and thus require fitting models that account for Ks, BM1 and K2 (weak association model). These results have been experimentally tested with serum proteins and IgG up to 120 mg/ml. Because a typical clinical dose of mAb is 50-200 mg/ml, these results have relevance for biophysical understanding of concentrated therapeutic proteins. Many therapeutic peptides, cytokines and proteins are attached to large linear and branched polyethylene glycol (PEG). PEG modification increases in vivo half-life while significantly altering osmotic pressure and solution nonideality. We will present results for a 40 kD branched PEG-modified HSA, which justifies the range of values tested (Ks = 66 ml/gm, BM1 = 96 ml/gm).

Dealing with Strong Non-Ideality in Self-Association: Lessons Learned From Teduglutide

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The small peptide teduglutide (3.5 kDa, used in treating short-bowel syndrome) strongly self-associates, with a weight-average molar mass rising above that of tetramer near 2 mg/mL. As it associates its alpha-helix content increases substantially, and the near-UV CD spectra indicate the associated state has a stable tertiary structure. We believe this peptide exhibits a concerted association to form pentamers, and then at concentrations near 10 mg/mL those pentamers weakly dimerize to form decamers. The association however is also strongly non-ideal even at concentrations in the low mg/mL range, which made determination of the binding stoichiometry and energetics rather difficult despite using modern data analysis methods and despite having both sedimentation equilibrium and sedimentation velocity data (up to ~15 mg/mL and ~2 mg/mL, respectively). Some lessons learned from this system will be discussed, as well as current limitations regarding treatments of the non-ideality.

Analytical ultracentrifugation reveals poly acidic or basic amino acids sequence alters protein

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Analytical ultracentrifugation has been utilized to capture the motion of macromolecules in centrifugation field, and is widely applied in assessing protein properties in solution, especially sedimentation coefficient, stokes radius, molecular weight, and hydrodynamic and thermodynamic parameters. Utilizing analytical ultracentrifugation, we analyzed the impact of poly acidic amino acids sequence on SnRK2.6's hydrodynamic properties. Chimeric proteins are engineered which combine full length PYL10 and c-terminal poly acidic amino acids sequence of SnRK2.6(333-362) or PDI(441-491), other chimeric proteins are engineered with SnRK2.6(1-332) and poly acidic amino acids sequence(DEDEDE) or poly basic amino acids sequence(KRKRKR). Compared with size exclusion chromatography(SEC) and static light scattering (SLS), we demonstrate addition of poly acidic or basic amino acids sequence leads to changes of Stokes Radius, axial ratio, friction ration, and decrease of elution volume on size exclusion chromatography.

HullRad: a fast, new tool for prediction of hydrodynamic parameters from structure

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Hydrodynamic properties are useful parameters for estimating the size and shape of proteins and nucleic acids in solution. The calculation of such properties from structural models informs on the solution properties of these molecules and complements corresponding structural studies. Here we report, to our knowledge, a new method to accurately predict the hydrodynamic properties of molecular structures. This method uses a convex hull model to estimate the hydrodynamic volume of the molecule and is orders of magnitude faster than common methods. It works well for both folded proteins and ensembles of conformationally heterogeneous proteins and for nucleic acids. Because of its simplicity and speed, the method should be useful for the modification of computer-generated, intrinsically disordered protein ensembles and ensembles of flexible, but folded, molecules in which rapid calculation of experimental parameters is needed. The convex hull method is implemented in a Python script called HullRad. The use of the method is facilitated by a web server and the code is freely available for batch applications.

Emulsification of ultra-flexible microemulsions (UFME) triggered by soft Centrifugation

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In ternary systems containing a hydrotropic co-solvent and two slightly miscible solvents, the phenomenon of weak dynamic aggregation far from the critical point favors the presence of weak aggregates stabilized by a slight accumulation of the hydrotrope between water-rich and oil-rich domains. The balance of solvation effects and entropy results in a preferred size of these UFME. Very often, but not always, a metastable emulsion can be obtained either by adding water, or by decreasing the temperature, or by soft-centrifugation to a composition of a single phase being close to that for the phase separation.

We use the well-studied system water-ethanol-octanol, with and without a dye (Nile red). Close to the single to two-phase boundary in this ternary system, separation by centrifugation is fast and efficient. The solute octanol is experiencing sedimentation in the centrifugal field as a dynamic aggregate and not as a molecule. Effects due to preferential solvation changing the effective solute density as discovered by Piazza are also present but orders of magnitude more intense than in the case of nanoparticles.

We describe the first series of experiments performed in this pre-ouzo region: Emulsification appears at the bottom of the tube, showing the influence of gravity. Moreover, after separation by soft centrifugation, either common a meniscus or a very peculiar turbid zone separates an oil-rich and a water-rich phase, with distribution of the hydrotropes and the model solutes (Nile red dye).

In this contribution, we describe the sedimentation equilibrium as well as sedimentation velocity experiments that illustrate this giant separation efficiency of soft centrifugation (3000 g only). These effects can be qualitatively understood and explained using a Flory-Huggins solution expression of the molecular interactions and thermodynamics involved. Using the approach of Jean Perrin allows disentanglement of the collective effects producing this efficient separation of small molecules in low centrifugal fields. This provides information about the free energy of both ternary weakly associated UFME in the presence of small solutes (> 300 Da) and much heavier molecules such as proteins or latex particles that need to be separated.

Analytical Ultracentrifugation for Understanding the Role of Cluster Nature in the Seed-Mediated Growth of Gold Nanocrystals

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Colloidal noble metal nanoparticles possess novel physical-chemical properties, which have found uses in electronics, biomedicine, energy harvesting or catalysis among other applications [1-3]. The emergence of the unique optical, electrical and magnetic properties is caused the reduction of the metal size to the nanoscale. Importantly, at this level, the shape of the ANCs play a key role in determining such properties and it is defined by the nature of the small atom aggregates or clusters formed in the initial stage of the synthesis [4].

Herein, multiwavelength analytical ultracentrifugation is exploited to investigate the role of the nature of atom clusters in the growth of anisotropic gold nanocrystals via a seed-mediated method. By controlling parameters such as the speed of reactant mixing, temperature or aging of the solutions, the size distributions and nature of the clusters can be tuned and thereby the outcome of the gold nanoparticle growth. Then, the final shape and size distribution and spectrum of the obtained gold nanocrystals is analysed and correlated with the nature of the seminal clusters previously characterized via analytical ultracentrifugation [5-7].

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Accessing core-shell properties and functionalization of nanoparticles by means of analytical ultracentrifugation

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Dispersion stability and functional properties of nanoparticles are significantly driven by surface properties. Small nanoparticles have to be considered as core–shell systems when dispersed in a liquid since a solvation layer and stabilizer shell will contribute to the particle's hydrodynamic diameter and effective density. Analytical ultracentrifugation (AUC) possesses great potential for multidimensional particle characterization.[1] As it allows capturing the sedimentation and diffusion properties of nanoparticles, it is further capable of assessing particle functionalization.[2]

Our first example will focus on the characterization of surface anisotropic particles comprising silver metal patches coated onto silica core particles, also referred to as patchy particles. So far, characterization of such particles has been time consuming as it required electron microscopy (EM) and tedious counting. We will show that AUC combined with multiwavelength detection (MWL-AUC [3-5]) provides multidimensional access to patchy particles. The distribution of patch mass on the core can be accurately followed via the sedimentation coefficient. Moreover, extinction spectra can be measured by MWL-AUC and linked to hydrodynamic properties. When relating data to finite element modelling and EM, information on the distribution of patch thickness and coverage is available. This allows for the targeted synthesis of patchy particles because samples can be characterized with high throughput.

In our second example, we will show that sedimentation analysis can be used to study the dynamic interaction between polystyrene or silica cores and microgels. It is demonstrated how the concentration dependent functionalization and bridging can be resolved by means of sedimentation coefficient distributions. This is a tremendous step forward for the analysis of such microgel-particle structures as they can be directly studied in the solution environment.

The presented developments significantly extend the capabilities of AUC with respect to particle characterization and are therefore of high importance for functional particle systems.

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Analysis of Ti/NbCN Nanoparticles Extracted from Microalloyed Steels by SV-AUC

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Microalloyed steels find applications in numerous industrial sectors for the production of structural parts due to their compelling mechanical properties and good weldability. Such steels contain small amounts (< 0.5 wt%) of niobium, titanium, vanadium, or combinations of the three as alloying elements. If the parameters during steel production are adequate, they lead to the formation of desirable precipitates that contain titanium, niobium, vanadium, carbon, and/or nitrogen. The microstructure and hence, the mechanical properties of microalloyed steels depend upon precipitate size distribution. Analysing the carbonitrides particle size distribution is therefore of great interest for material engineering.

Particle size distributions (PSDs) in steel are traditionally assessed by investigating electron transparent foils or carbon replica using electron microscopy. Such techniques only give access to a small number of particles and hence may not be representative of the sample volume. We use an alternative, volume-based method to investigate the particle size of carbonitrides contained in microalloyed steels. The particles are extracted from a steel sample with a representative volume by chemical dissolution of the iron matrix using sulfuric acid. After purification of the obtained suspension Analytical Ultracentrifugation (AUC), Field-Flow-Fractionation (FFF), Transmission Electron Microscopy (TEM), and Scanning Transmission Electron Microscopy (STEM) are employed for characterization.

Here, we evaluate the suitability of Sedimentation Velocity Analytical Ultracentrifugation (SV-AUC) for determination of the extracted particles PSD by least-squares direct boundary modelling. We discuss the assumptions required to transform the extinction weighted PSDs obtained by AUC and FFF into number weighted PSDs. These are then compared to the number weighted PSDs provided by TEM and STEM analysis in order to validate the data generated by SV-AUC.

Concentrated colloidal nanoparticles in Analytical Ultracentrifugation

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The sedimentation of colloidal nanoparticles is of great interest to both condensed matter physics and colloidal chemistry. Nanoparticles can sediment until a sedimentation-diffusion equilibrium state is reached. From the equilibrium concentration profile, important thermodynamic information of the particles can be retrieved. The classic example is provided by Perrin who determined the Boltzmann constant by measuring the sedimentation-diffusion profile of pollen particles under the natural gravity. However, the sedimentation of concentrated nanoparticles with a much broader practical importance still lacks careful studies so far.

In this presentation, the sedimentation of multiple colloidal nanoparticles at a concentration as high as 30 vol% is studied using the combined techniques of fluorescence labeling, refractive index matching and multi-wavelength analytical ultracentrifugation. The theoretical studies are also conducted to understand the experimental data. We will mainly focus on the following cases: the sedimentation of monodisperse and the mixture of (1) hard sphere like1-2 and (2) charge stabilized nanoparticles3. The experimental and theoretical work in the presentation may be intriguing to the research of biomolecules, such as proteins and DNA molecules in highly concentrated environments, which may help us understand how these molecules work inside crowded cells.

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Ligand interactions of amyloid-β protein studied by analytical ultracentrifugation in concert with microscale thermophoresis

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Amyloid-β peptide (Aβ) is an intrinsically disordered protein (IDP) associated with Alzheimers disease. The structural flexibility and aggregation propensity of Aβ pose major challenges for elucidating the interaction between Aβ monomers and ligands. All-D-peptides consisting solely of D-enantiomeric amino acid residues are interesting drug candidates that combine high binding specificity with high metabolic stability. Here we characterized the interaction between the 12-residue all-D-peptide D3 and Aβ42 monomers, and how the interaction influences Aβ42 aggregation. We demonstrate for the first time that D3 binds to Aβ42 monomers with submicromolar affinities. These two highly unstructured molecules are able to form complexes with 1:1 and other stoichiometries. Further, D3 at substoichiometric concentrations effectively slows down the β sheet formation and Aβ42 fibrillation by modulating the nucleation process. The study provides new insights into the molecular mechanism of how D3 affects Aβ assemblies and contributes to our knowledge on the interaction between two IDPs.

Modelling APOBEC3-ssDNA bound complexes using small-angle X-ray scattering

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The APOBEC3 (A3) protein family are cytidine deaminases that act as double-edged swords. On one side they attack retroviruses and other pathogens by inducing hypermutation through deamination of cytidine residues on single-stranded DNA [1]. While, several APOBEC3 family members, particularly A3B and A3G, are utilised by viruses and cancer cells to increase the rates of mutagenesis, escape adaptive immune responses, and become drug resistant [2, 3]. Inhibition of A3 proteins may therefore be used to augment existing anticancer and retroviral therapies, and we have synthesised and characterised the first inhibitors to A3 enzymes [4,5]. Previous work has shed some light on how single A3 catalytic domains bind to single-stranded nucleic acid substrates (ssDNA) [6,7]. However, the structures of ssDNA complexes with the full-length two-domain A3 enzymes remain elusive. We have used small-angle X-ray scattering (SAXS) to elucidate the structural changes upon binding of ssDNA to A3 proteins. We obtained envelope models of the catalytic domain of A3B-ssDNA inhibitor complex, which exhibited slight changes in the radius of gyration (R_g) upon complex formation. Additionally, the putatively dimeric form of the A3B catalytic domain provided SAXS envelopes and structural models consistent with a dimeric form. However, upon binding of ssDNA the dimer interface disassembles into a monomeric A3B-ssDNA complex. We have also established a SAXS structural model for a two-domain A3G-ssDNA complex, which revealed large structural changes upon complex formation. This constitutes the first full-length A3-ssDNA model and will provide a platform for further structural studies and development of A3 inhibitors.

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Models for the site-specific treatment of cooperative ligand binding by proteins

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The non-covalent binding atoms or small molecules by proteins is central to cellular biology. Proteins and protein complexes will frequently bind multiple ligands in a cooperative fashion. The linkage of binding events provides a mechanism for information transmission and biological regulation. Hence characterizing cooperative ligand binding by proteins is of profound importance.

Ligand binding events can be modeled at differing levels of detail. Stoichiometric models consider proteins with the same number of ligands bound as equivalent. Site-specific models discriminate between the different ligand binding sites - a prerequisite for explaining binding phenomena at the molecular level. Conformational models additionally describe the shifts in protein conformational ensembles or the changes in conformational dynamics that are associated with ligand binding. Conformational models speak to the actual mechanism of cooperativity, but can be difficult to specify correctly. Equilibrium thermodynamic modeling of ligand binding, at the site-specific level, occupies the critical middle ground. It allows us to understand the energetic interactions between binding processes, and provides a platform on which mechanistic models can subsequently be developed.

We describe a formalism for developing site-specific ligand binding models for systems of arbitrary complexity. The equilibrium thermodynamic model is parameterized in a hierarchical and non-redundant fashion, employing a binary vector notation to denote the states and parameters of the model. This allows a closed form expression for the binding polynomial to be written. The notation and parameterization facilitate the straightforward treatment of protein symmetry, and the incorporation of simplifications (e.g. Nearest Neighbor approximations) which are usually critical to practical applications. These models can be used to treat both homotropic and heterotropic ligand binding and can produce the full repertoire of cooperative behavior observed for ligand-binding proteins. They are broadly applicable, because they assume nothing about the physical location of the sites, or the mechanism of any linkage between them. Hence they can be used to model both allosteric and non-allosteric interactions between sites.

Sedimentation velocity studies of two salivary glycoproteins: possible structure-function effects of food flavour

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Almost 90% of aroma and taste compounds in food are not available to be perceived due to the rapid ingestion of food, this is known to be dependent on the chemical composition of the food material. Therefore aroma and taste rebalancing is key when reformulating foods for reduced fat, sugar, or elevated levels of protein. Furthermore, for flavour to be perceived it needs to dissolve in the aqueous saliva coating the oral-nasal mucosa, we believe that the chemical composition of this saliva has a significant impact on its ability to act as an effective solvent for these flavour compounds. Whilst saliva has a complex proteome, two of its most abundant macromolecules, submaxillary mucin and salivary α-amylase, were used to understand some of the interactions underpinning the release of aroma and taste compounds in vitro, as these glycoproteins are important in its formation of salivary microstructures in mouth. We used sedimentation velocity analytical ultracentrifugation in combination with gas chromatography-mass spectrometry to explain these interactions. Findings from these experiments were able to add to our current understanding of the hydrodynamic behaviour of mucin and α-amylase during oral processing, such as acid and salt effects on their structure and how these may affect aroma release. Sedimentation velocity studies suggested interactions between specific aroma compounds (e.g. aldehydes) and the two salivary glycoproteins, in both dilute food systems (less than 0.01 mg/ml), and high concentration (>10 mg/ml) flavour compounds (e.g. e-cigarettes). We therefore suggest new potential applications of AUC in the food and biopharmaceutical industries.

The repressor, depressor of sialic acid catabolism

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Bacteria carefully regulate metabolic processes to efficiently colonise and persist within the human host. One mechanism bacteria employ is gene regulation. In *Escherichia coli*, the catabolism of sialic acid is controlled by the GntR-type transcriptional regulator NanR, but the mechanism of gene regulation is unknown. We first demonstrate that NanR binds as a dimer to a total of three direct GGTATA repeats that make up the DNA recognition site, forming a hexameric assembly. Interestingly, we found this binding is cooperative and demonstrate it is mediated by a unique N-terminal extension, likely through protein-protein interactions. To understand how DNA-binding is attenuated by the effector, we solve the co-crystal structure of *E. coli* NanR in the presence of *N*-acetylneuraminic acid to 2.1 Å, supporting the hypothesis that this is the effector molecule, and identify a metal-binding motif that coordinates zinc. The structure is asymmetrical with *N*-acetylneuraminic acid and zinc only present in one monomer, informing the molecular details of the conformational change that occurs following binding to attenuate DNA-binding activity. Notably, we report the structure of the NanR-DNA complex to 3.9 Å using cryo-electron microscopy, and the first structural evidence of a multimeric assembly process within the GntR superfamily. Together, our results give the first molecular insight into the mechanism of the NanR-DNA interaction in *E. coli*, which enhances our understanding of sialic acid gene regulation in bacteria.

Biophysical characterisation of novel Flavivirus Antivirals That Target Host Nuclear Transport

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Dengue virus (DENV) threatens almost 70% of the world's population, with no effective vaccine or therapeutic currently available. A key contributor to infection is nuclear localisation in the infected cell of DENV nonstructural protein 5 (NS5) through the action of the host importin (IMP) α/β1 proteins. Using a range of microscopic, virological and biophysical approaches we show that the small molecules GW5074 and ivermectin have anti-DENV action through their novel ability to inhibit the NS5–IMPα/β1 interaction in vitro as well as NS5 nuclear localisation in infected cells. Strikingly, the small molecules not only inhibit IMPα binding to IMPβ1, but can dissociate preformed IMPα/β1 heterodimer, through targeting the IMPα armadillo (ARM) repeat domain to impact IMPα thermal stability and α-helicity, as shown using analytical ultracentrifugation, thermostability analysis and circular dichroism measurements. Importantly, GW5074 has strong antiviral activity at low μM concentrations against not only DENV-2, but also zika virus and West Nile virus. This work highlights DENV NS5 nuclear targeting as a viable target for anti-flaviviral therapeutics.

Determination of two-dimensional size distributions of plasmonic nanoparticles via the optical back coupling method

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Since nanoparticles (NPs) exhibit a large variety of properties, the analysis of shape, size and density is essential. However, the combined analysis of these parameters is a major challenge. Analytical ultracentrifugation (AUC) is a powerful and highly accurate tool for the multidimensional analysis of NPs. The combination of AUC analysis and additional independent measurement techniques like the scanning mobility particle sizer can only partially resolve multidimensional information.[1,2] In order to be able to determine true two-dimensional size distributions (2DSD)[3] coupled complementary information needs to be acquired. Analytical ultracentrifugation equipped with a multiwavelength detector (MWL-AUC) is able to provide this information as it combines UV/VIS (240 to 1000nm) spectroscopy with fractionation in a centrifugal field. From the experiments, sedimentation coefficients, which depend on the particle geometry, the density of particles as well as the solvent, can be calculated. Optical properties can thus be directly correlated with the sedimentation behavior.

In the case of plasmonic particles, the optical spectra include information on the composition as well as on the geometry. MWL-AUC provides the combined information of sedimentation coefficients and optical spectra which can be used to derive truly 2DSD in one single experiment via optical back coupling (OBC). The OBC-method uses the determined sedimentation coefficient distributions at various wavelengths to extract optical spectra. The spectra are analyzed in terms of geometrical information, which is then used together with the original sedimentation coefficient to determine the 2DSD of e.g. gold nanorods.[4] From this 2D-dataset any desired one- and two-dimensional distribution (length, diameter, aspect ratio, area and volume) can be calculated.[3,4] This fast and statistically reliable approach is very promising, as usual 2DSD have to be acquired using tedious SEM/TEM analysis. Additionally to size analysis, the OBC-method can be used to validate optical models and to determine supplementary hydrodynamic and optical parameters.

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Hydrodynamic analysis of co-polymers with a gradient structure: solution properties, molar mass and conformation

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Gradient copolymers represent rather new class of polymeric macromolecules that are characterize by a gradual change in their monomer composition along molecular chains. The asymmetric distribution of monomers within each chain may result in very specific and unique properties of such polymer molecules in comparison to their "regular" analogues. However, in spite of large and growing interest in synthesis and analysis of gradient copolymers they are currently far from being fully understood, especially in terms their solution behavior and conformation. From this point of view, we should think of understanding the very basic structure property relationships between the chemical structure and corresponding molecular behavior and conformational properties in solution, which can then be attributed to very specific physical-chemical properties of a polymer based supramolecular structures/materials. In the here presented research we have studied tapered multiblock copolymers having polystyrene and polyisoprene as monomer units. Polystyrene and polyisoprene are well known polymers, yet their combination in a block gradient like composition of a polymer chain results in promising mechanical characteristics. The macromolecules have gradient composition within one block which molar mass as well as number of blocks were varied in a wide range (from 1 to 10). The overall molar mass range was from ~10 kDa to 400 kDa, the polymers were studied in toluene. To overcome common characterization pitfalls we applied combined analytical approach that relies on first principle measurements such as analytical ultracentrifugation, intrinsic viscosity, and translation diffusion. The consistency of the experimental data was verified via the hydrodynamic invariant concept. All basic solution and molecular characteristics were determined including primary conformational parameters – the Kuhn segment length and the diameter of a polymer chain.

Characterization of Linear and Cyclic Polystyrene Sulfonates using Analytical Ultracentrifugation

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Polystyrene sulfonate (PSS), as one of the most important categories of polyelectrolytes, has received increasing attention due to its great potential in the applications of energy- and biomedical-related fields. However, most of previous studies have only focused on linear PSS and its derivatives, but little attention was paid to non-linear topological PSSs. So far, the synthesis of non-linear PSSs with well-defined structures is still a challenging task, and the main obstacle lies in the stability issue of functional chemical linkages during sulfonation process of polystyrene (PS) precursors. Herein, by rationally designing the chemical structure of functional linkage, we introduce a versatile and efficient strategy for the preparation of topological PSSs. By using two sets of PSS samples with varied molar masses, the scaling relations between molar mass and sedimentation coefficient were established for both linear and cyclic PSSs.

AUC analysis of chitosan and aminocellulose for use in archaeological wood conservation

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Archaeological wood can be difficult to conserve. Previous treatment and the presence of iron can make conservation more difficult. Cationic polymers may prove beneficial to conservation as they may help chelate metal ions and mop up some acid. However, before wood treatment it is important to establish the molecular weight of the polymer and its distribution. This study focused on three potential consolidants: (1) Chitosan Kit-Nor depolymerised by ~60% yielding an Mw of ~5-6kDa determined by SE. Successful infusion into wood was observable after modification to add a silica containing group. (2) An aminocellulose chosen was (AEA) because of our previously reported studies demonstrating self-association/assembly (Nikolajski et al., 2014) We also find self-association with this AEA of a lower degree of substitution of ethenediamine. AEA could not be observed on IR or SEM due to over lapping IR peaks with cellulose and the way it probably coats the wood cell, however weight gain was observed. (3) Finally, a modified aminocellulose was also examined which had a terminal hydroxyl on the C-6 side chain (HEA). This was also found to self-associate, but it was only partially reversible. HEA similarly could not be observed on SEM or IR, however weight gain was observed and the anti-shrink efficiency was improved. HEA appeared to consolidate the wood well. Chitosan appeared successful in consolidation of wood but there is concern over the requirement of acetic acid for dissolution. Aminocellulose appears particularly promising for conservation but still requires more AUC research into interaction with molecules in the cell wall and more conservation research before it can be applied to the precious artefacts.

A proteomic screen of neuronal cell surface molecules reveals IgLONs as structurally conserved interaction modules at the synapse

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The brain has a highly complex yet very precise pattern of neuronal connectivity. Genetic evidence indicates that over 100 neurological and psychiatric diseases are likely caused by genetic abnormalities of specific proteins located on the neuronal surface. This proteins specify the formation of new synapses, their maturation and turnover. Although the identity of these proteins is known, their network of interactions remains largely untapped. Using an ELISA-based proteomic screen we have identified 200 interactions, 89 of which were not previously published. Among these interactions, we find that the IgLONs, a family of five cell-surface neuronal proteins implicated in various human disorders, interact as homo- and heterodimers. Using analytical ultracentrifugation, protein crystallography and small angle X-ray scattering, we reveal their interaction patterns and report the dimeric crystal structures of Neurotrimin (NTRI), IgLON5, and the neuronal growth regulator 1 (NEGR1)/IgLON5 complex. We show that IgLONs maintain an extended conformation and that their dimerization occurs through the first Ig domain of each monomer and is Ca2+ independent. Cell aggregation shows that NTRI and NEGR1 homo- and heterodimerize in trans. Taken together, we report 89 unpublished cell-surface ligand-receptor pairs and describe structural models of trans interactions of IgLONs, showing that their structures are compatible with a model of interaction across the synaptic cleft.

Towards studying nano-scale structures of Flaviviral non-coding RNAs-host protein complexes.

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RNA-protein interactions play crucial roles in a number of biological processes. To understand mechanisms underpinning such process, structural information on biomolecular complexes are essential. However, the experimental determination of biomolecular complexes that provides information on the position, orientation, and interactions of individual domains is often time-consuming and in many cases not possible. As a result, complete high-resolution structures are not yet available for many complexes that are crucial for numerous fundamental cellular processes. An emerging alternative to high-resolution structural techniques is to employ a hybrid approach that combines low-resolution shape information about macromolecules and their complexes from hydrodynamic and SAXS methods, with high-resolution structures (where available), and with computational modeling to obtain atomic-level models. Our laboratory has established such an approach and has used for a variety of viral RNAs as well as their complexes involving RNA-protein complexes. We will discuss the recent advances Flaviviral RNA-host protein interactions.

E. coli primase and DNA polymerase III holoenzyme are able to bind concurrently to a primed template during DNA replication

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During DNA replication in E. coli, a switch between DnaG primase and DNA polymerase III holoenzyme (pol III) activities has to occur every time when the synthesis of a new Okazaki fragment starts. As both primase and the chi subunit of pol III interact with the highly conserved C-terminus of single-stranded DNA-binding protein (SSB), it had been proposed that the binding of both proteins to SSB is mutually exclusive. Using analytical ultracentrifugation with fluorescence detection and a replication system containing the origin of replication of the single-stranded DNA phage G4 (G4ori) saturated with SSB, we tested whether DnaG and pol III can bind concurrently to the primed template. We found that in the absence of ribonucleotides only one primase binds specifically to G4ori, whereas two molecules bind in their presence. The addition of pol III does not lead to a displacement of primase, but to the formation of higher complexes. Even pol III-mediated primer elongation by one or several DNA nucleotides does not result in dissociation of DnaG. About 10 nucleotides have to be added in order to displace one of the two primase molecules bound to SSB-saturated G4ori. The concurrent binding of primase and pol III is highly plausible, since even the SSB tetramer situated directly next to the 3 terminus of the primer provides four C-termini for protein-protein interactions.

Tracking polyglutamine and polyalanine aggregates in cells using fluorescence-detected analytical ultracentrifugation

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Protein aggregation into intracellular inclusions is a key feature of many neurodegenerative diseases, yet how they are formed in cells is enigmatic and defining the process by which they form remains a challenging task due to a lack of robust techniques for their isolation and quantitation. We have used fluorescence-detected sedimentation velocity analysis to define and compare the heterogeneity and flux of poly-amino-acid aggregates expressed in mammalian cells under non-denaturing conditions. We demonstrate that this approach can provide significant insight into aggregation formation and kinetics in disease states.

How does Rubisco activase activate Rubisco?

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Rubisco is the most abundant and arguably the most important enzyme in the world, catalysing the incorporation of carbon dioxide into the biosphere. Accordingly, much research is currently aimed at improving photosynthesis through the improvement of Rubisco. Unfortunately, Rubisco tends to accumulate a variety of inhibitors in the active site, slowing its catalytic ability. In order to sustain high rates of photosynthesis, plants use an ancillary protein, called Rubisco activase, which releases inhibitors from the Rubisco active site. Despite the importance of this interaction in maintaining plant growth, and the fact that much work has been done to characterise the interaction of Rubisco with other proteins, little is known about how Rubisco actually interacts with Rubisco activase. In order to better understand this interaction, we have been studying the arrangement of Rubisco activase in solution from a variety of different species, including tobacco, spinach, spruce, cotton and creosote. Intriguingly, Rubisco activase forms a diverse range of oligomeric species, with some variants forming hexamers in solution, similar to other members of the AAA+ family of proteins, while other species form a range of different oligomers in solution, ranging from monomers to >10 subunits. Our current work aims to solve the structure of Rubisco activase in solution, in order to gain a better understanding of how it can function.

What happened to the HIV capsid? A tale of armored viruses, goldilocks zones, molecular staples and traitorous co-factors

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Background: HIV-1 infection is an ongoing medical crisis. Efforts to develop drugs to combat infection have been challenged by the adaptability of the retrovirus. Rational drug discovery strategies depend on a deep understanding of the retroviral life-cycle as opposed to the hit-or-miss approach taken by high-throughput screening. In other words, uncovering the details of the HIV-1 infection process –down to specific viral protein / mammalian host protein interactions will provide the foundation of rational drug discovery. This is where we have focused our efforts. There are several crucial steps in the life cycle of HIV-1 which occur after injection of the HIV-1 genome into the host cell. The retroviral RNA is reverse transcribed into cDNA – which must be then integrated into host's genome. The virus utilizes the host cell's cytoskeletal machinery to transport the HIV-1 capsid to the host nucleus. Each of these steps in the viral life cycle and each point at which a host cell protein is recruited by the invading virus is a potential drug target. Here, we discuss the interaction of mammalian CPSF6 protein with the capsid p24 protein which forms the armored sheath that encapsulates the viral genome. CPSF6 is known to have HIV-1 restriction properties.

Results and Conclusions: We have uncovered a binding interaction between CPSF6 and the HIV-1 capsid hexameric protein which forms a "molecular bridge" across adjacent units p24 monomer units. This leads to over-stabilization of the capsid – and restriction of viral infection. Interestingly, the interaction patch on the viral capsid which binds to CPSF6 was also found to be targeted by drug candidates. Thus, understanding the fate of the HIV-1 capsid promises to be very useful in developing new antiretroviral drugs.

Methods: We have used a combination of X-Ray crystallography to obtain structural information about both systems, analytical unltracentrifugation and fluorescence polarization spectroscopy for binding studies and NMR spectroscopy for dNTPase assays in the SAMHD1 system

High concentration AUC analysis of biopharmaceuticals

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AUC is widely used as an orthogonal technique to confirm the aggregation content routinely measured by size-exclusion chromatography. In order to optimize accuracy and precision it is typically employed at moderate concentrations around 0.5 mg per ml in a simple PBS-like buffer. The aim is to optimize the sample composition in order to meet the requirements of the technique best. However, the advent of high concentration formulations of biopharmaceuticals at 100 mg per ml or even higher and many of them self-buffering, renders this approach questionable. Furthermore, predictions on long-term stability and aggregation propensity are only valid if the intact system, the undiluted protein in its native formulation is analyzed. The recent development of new fitting algorithms for non-ideal sedimentation in Sedanal and sedfit allows the determination self and cross-sedimentation terms as well as the non-ideality parameters kS and kD. In combination with 3D printed centerpieces this allows the accurate description of protein solutions up to 100 mg per ml and even the discovery of previously unrecognized self-association (Chaturvedi et al. 2019). We have explored the capacity of AUC to accurately quantify the aggregation content of a marketed antibody formulation (Humira) using a model protein mimicking an antibody dimer. While hydrodynamic and thermodynamic non ideality affects the analysis of globular non-glycosylated proteins and even monoclonal antibodies only at concentrations higher than 2 mg per ml, these effects can be quite prominent for proteoglycans even far below these concentrations. During the formulation development of Lubricin, a synovial proteoglycan, AUC proved to be particularly useful to explain problems like filter clogging and determination of the molecular mass. We demonstrate how SV and SE AUC can be used to guide early development of biopharmaceutical formats other than mAbs, especially those that exhibit strong non-ideality at even low concentrations.

Metal-dependent dynamic equilibrium: A mechanism for regulation of the Plasmodium M17 aminopeptidases?

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M17 leucyl aminopeptidases are metal-dependent hexameric enzymes found in almost all kingdoms of life. A major function of M17 aminopeptidases is the cleavage of single amino acids from peptide substrates. The M17 aminopeptidases from malaria-causing parasites Plasmodium falciparum (Pf-M17) and Plasmodium vivax (Pv-M17), are postulated to liberate free amino acids from short haemoglobin peptides for use in parasite protein production. Pf-M17 has been validated as a potential drug target for the design of new anti-malarial treatments, and high sequence identity between Pf- and Pv-M17 suggest Pv-M17 may also present as an attractive target.

To further understand these aminopeptidases, we investigated the relationship between oligomeric state and aminopeptidase activity, and the role metal ions play in these different molecular mechanisms. We found both Pf- and Pv-M17 exist in a metal-dependent equilibrium between an active hexamer and inactive small oligomers. This equilibrium is dependent on both metal ion concentration and identity. To further explore the impact of metal ion environment on aminopeptidase activity, we kinetically characterised Pf- and Pv-M17 activity against two distinct substrates and determined that the metal ion environment can moderate enzyme substrate specificity and catalytic activity. Mutation of two of the metal-binding residues resulted in compromised catalytic activity and disruption of the metal-dependent equilibrium system. During the Plasmodium life cycle the identity and concentration of metal ions fluctuates. Therefore, we propose parasites may be utilizing the M17 metal-dependent dynamic equilibrium as a biological regulator of haemoglobin proteolysis in parasites.

Characterisation of high molecular weight hop proanthocyanidins using Analytical Ultracentrifugation

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We report the novel application of Analytical Ultracentrifugation (AUC) to characterise – without the need of a separation matrix or membrane - the polymeric proanthocyanidin fraction extracted from hops. Extraction of hop samples with 70% acetone (aq) followed by a C-18 Solid Phase Extraction clean-up yielded polyphenolic fractions for AUC analysis. Sedimentation velocity experiments demonstrated the presence of discrete molecular weight bands of proanthocyanidins, as opposed to a continuous distribution of molecular weights. There were 4 such bands for Saaz hop (0.15, 1.1, 2.7 and 4.4 S) and 3 bands for Magnum (0.15, 1.6 and 3.0 S). The method resulted in a reproducible c(s) distribution for replicate runs of the same extract and for extracts prepared from different samples of the same hop variety. Sedimentation equilibrium experiments were then used to fit molecular weight distributions to c(M) data for the same samples. Thus, we report for the first time the presence of polymeric proanthocyanidins in hops with molecular weights of up to 100 kDa in Saaz hop (or up to 56 kDa in Magnum). This represents the first application of AUC to characterise complex fractions of polyphenolics extracted from botanical sources and the methodology developed should find wider application in the study of this diverse and bioactive class of compounds.

Single molecule versus bulk detection: Interferometric light scattering (iSCAMS) and AUC applied to study protein-protein interactions

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Measurements of biomolecular interactions are crucial for understanding the mechanisms of the biological processes they facilitate. Cell metabolism, regulation of cellular process, and the assembly of intracellular structures are all examples of molecular interactions of different strength and complexity. Despite the fact that several new methods (MST, BLI) for studies of molecular interactions were recently developed, (AUC) remains one of the most powerful techniques in the biophysical arsenal. AUC can be applied to study interactions with affinities ranging from sub-nanomolar to millimolar and involving particles with molar masses spanning six orders of magnitude (from kDa to GDa). A particular strength of the AUC, compared to the SPR, ITC, and other widely used techniques, is its ability to obtain size distributions of molecules in solution. This provides information on the molecular composition of the system under study and helps identify complexes formed during molecular interactions. Consequently, AUC is used to study both the stoichiometry and the affinity of interactions.

A recently developed single molecule method, the interferometric light scattering (iSCAMS), provides mass distributions of native molecules in solution (Young et al., 2018). In comparison with the AUC, iSCAMS requires a very small amount of material and is very fast with a typical experiment time of only 60s. Here we applied iSCAMS and AUC to measure the affinities of biomolecular interactions in the antigen-antibody system to compare the advantages of both techniques. We have demonstrated how the high mass resolution of iSCAMS allows the user to quantify the 1:1 and 1:2 complexes formed during the interaction. Affinity values obtained from the single molecule iSCAMS and from the AUC measurements have been further validated with the SPR assay.

Our data show the high accuracy and reach information content of the iSCAMS measurements. iSCAMS does not require labeling and is able to quickly probe populations of free and bound protein states in solution. These advantages, combined with simple and fast measurements as well as a low sample consumption makes iSCAMS a new preferred method to measure strong protein-protein and protein-DNA interactions.

Young et al., Quantitative mass imaging of single biological macromolecules, Science 360, 423-427 (2018)

Modern Approaches to the Detection of Ligand-Induced Protein Conformational Changes using AUC

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Conformational dynamics are crucial aspects of protein functioning, underpinning processes such as enzyme catalysis, signaling, and ligand binding. A particularly noteworthy category of protein dynamics is ligand-induced conformational change. In such changes, the unliganded protein, which exists in solution as a constellation of conformers, is induced by the binding of a small molecule to adopt a different, perhaps more circumscribed, set of conformers. Macroscopically, these changes can be observed as differences in bulk macromolecular properties, e.g. the radius of gyration and the sedimentation coefficient (or the"S-value"). As demonstrated over sixty years ago by Schachman and colleagues, Rayleigh interferometry coupled with analytical ultracentrifugation (AUC) in the sedimentation velocity (SV) mode is capable of detecting the small (ca. 1%) s-value variations that are expected from protein conformational changes. All of the pioneering work in the field was accomplished with older analytic models and slower, analog data-acquisition techniques. After scrutiny of simulations and model systems, we have arrived at a set of best practices for achieving the high data-acquisition and analytic precision necessary to detect protein conformational changes. Where appropriate, software solutions have been made available to facilitate experimental planning and analysis.

Structural and biophysical characterisation of the master regulator TRIM28

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TRIM28 (KAP1/TIF1B) has been labelled the 'enigmatic master regulator of the human genome' due to its involvement in a multitude of cellular processes including cell growth and differentiation, pluripotency, transcription repression, DNA repair and tumorigenesis. TRIM28 does not directly bind to DNA, rather it is recruited to the target genes by the Krab-ZFPs (Kruppel-associated box-containing zinc finger proteins) - the largest single family of transcription regulators in mammalian cells. Once recruited, TRIM28 acts as a scaffold and recruits effector proteins and protein complexes involved in repressive histone modification and de novo DNA methylation, ultimately leading to chromatin condensation and gene silencing. However, the mechanism of TRIM28-mediated silencing and the various protein-protein interactions that are fundamental to this process remain poorly understood.

TRIM28 belongs to the TRIM protein family with over 70 members in humans. They share a conserved N-terminal tripartite motif (TRIM), also known as the RBCC as it consists of a RING domain, one or two B-box domains and an antiparallel coiled-coil. Recent studies have highlighted the importance of TRIM protein self-assembly in their function.

To understand how Trim28 is recruited to a specific site we have undertaken structural and biophysical experiments to investigate the self-assembly of Trim28, identifying the domain responsible for self-association, determining the structure of this domain and designing mutants that block assembly. These mutants have then been used as a tool to characterise the interaction between Trim28 and the KRAB-ZFP proteins, determining the strength and stoichiometry of the interaction and using SAXS to define the binding site within the Trim28 RBCC.

Adenylosuccinate synthetase from *Helicobacter pylori*: biochemical and biophysical characteristics including comparison of C- and N-terminal His-tag variants

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There are two metabolic routes, which organisms can use to obtain purines, the indispensable building blocks of DNA and RNA - *de novo* and salvage pathways. Some organisms, including those, causing severe diseases, have only the salvage pathway [1]. Enzymes of this pathway are therefore good targets to disable growth and replication of such pathogens. We decided to obtain and characterize the adenylosuccinate synthetase (AdSS) from the *Helicobacter pylori* (HP) salvage pathway as a first step to design its potent, selective inhibitors.

Recombinant HP AdSS was purified to homogeneity using three chromatographic steps, however the yield and reproducibility of this procedure were rather poor. Analytical ultracentrifugation shows that the enzyme is a stable dimer even at a concentration below 1 μM, and in the absence of ligands, by contrast to its *E. coli* counterpart [2]. Optimal conditions for catalysis are 50°C and pH 7.0. Kinetic constants for the substrates (asparagine, inosine-5'-monophosphate, guanosine-5'-triphosphate) are comparable to those of enzymes from other bacteria. Natural antibiotic hadacidin strongly inhibits HP AdSS (K_i=0.19±0.02 μM) [3] but has almost no effect on the HP cell cultures growth. Attempts to identify active *in vivo* inhibitors are undertaken.

By characterizing the properties of the enzyme, we obtained one highly unpredictable result. In some conditions the specific activity depends on the HP AdSS concentration. Attempts to understand molecular mechanism responsible for this behaviour are in progress.

Crystals of the enzyme do not diffract good enough for the atomic-resolution X-ray structural studies. To test more crystallization conditions, we scaled-up the purification procedure by obtaining the C- and the N-terminal His-tag AdSS versions, and also AdSS with the thrombin cleavage site to remove His-tag after purification. Surprisingly, the His-tag variants seem to have some properties, including dimer-monomer equilibrium, different that their native counterpart.

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The solution structure of the human IgG2 subclass is distinct from those for human IgG1 and IgG4, providing an explanation for their discrete functions

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Human IgG2 antibody displays distinct therapeutically-useful properties compared with the IgG1, IgG3 and IgG4 antibody subclasses. IgG2 is the second most abundant IgG subclass, being able to bind human FcγRII/FcγRIII, but not to FcγRI or complement C1q. Structural information on IgG2 is limited by the absence of a full-length crystal structure for this. To this end, we determined the solution structure of human myeloma IgG2 (composed of two Fab regions and one Fc region) by atomistic X-ray and neutron scattering modelling. Mass spectrometry and AUC showed only a 2% range in mass and a single c(s) peak respectively, thus IgG2 polydispersity was low and did not preclude molecular structure analyses. Analytical ultracentrifugation disclosed that IgG2 is monomeric with a sedimentation coefficient $s_{20,w}$ of 7.2 S. IgG2 dimer formation was ≤ 5% and independent of the buffer conditions. Small-angle X-ray scattering in a range of NaCl concentrations and in light and heavy water revealed that the X-ray radius of gyration Rg is 5.2-5.4 nm, after allowing for radiation damage at higher concentrations, and that the neutron Rg value of 5.0 nm remained unchanged in all conditions. The X-ray and neutron distance distribution curves P(r) revealed two peaks, M1 and M2, that were unchanged in different buffers. The creation of >123,000 physically-realistic atomistic models by Monte Carlo simulations for joint X-ray and neutron-scattering curve fits, constrained by the requirement of correct disulfide bridges in the hinge, resulted in the determination of symmetric Y-shaped IgG2 structures. These molecular structures were distinct from those for asymmetric IgG1 and asymmetric and symmetric IgG4, and were attributable to the four hinge disulfides. Our IgG2 structures rationalize the existence of the human IgG1, IgG2, and IgG4 subclasses, and explain the receptor binding functions of IgG2. Steric clashes between the C1q domains and the FcγRI receptor with our IgG2 models explain why C1q and FcγRI do not bind to IgG2, while the absence of these clashes for the IgG2-FcγRIIIA complex showed that this interaction is permitted.

Reference: Hui, G. K., Gardener, A. D., Begum, H., Gor, J. and Perkins, S. J. (2019) J. Biol. Chem. 294, in press.

Unravelling the solution structures and stabilities of therapeutic antibodies with and without glycans

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The human immunoglobulin G class is the most abundant antibody in serum and comprises four subclasses IgG1-IgG4, each with two Fab regions connected to an Fc region through a hinge. Two Fc glycans are conserved in the five human classes, however their structural importance remains unclear. To address this question, we subjected two monoclonal IgG1 A33 and IgG4 A33 to a multidisciplinary structural and modelling strategy. Mass spectrometry demonstrated full deglycosylation using PNGase F. Analytical ultracentrifugation showed that the sedimentation coefficients s020,w of both IgG1 and IgG4 decreased from 6.45 S and 6.52 S by 0.16 and 0.27 S respectively after deglycosylation, indicating a slight elongation of their structures, and both subclasses were predominantly monomeric in their histidine buffer. X-ray solution scattering showed small increases of 0.07 nm and 0.03 nm in the X-ray radius of gyration Rg of IgG1 and IgG4 after deglycosylation, while this change was less apparent by neutron scattering for IgG1 and IgG4 in heavy water buffers. However the X-ray and neutron distance distribution curves P(r) revealed two peaks, M1 and M2, of which M2 was significantly shifted to larger values to indicate a greater separation between the Fab and Fc regions in deglycosylated IgG1. This shift was less apparent for IgG4. Atomistic scattering modelling revealed asymmetric solution structures for IgG1 and IgG4 with extended hinges, in which the effect of glycan removal was not detected. The thermal stability of glycosylated and deglycosylated IgG1 and IgG4 with and without glycans was investigated by monitoring the intrinsic fluorescence of the proteins to give melting temperatures. Both IgG1 and IgG4 exhibited two transitions. The first transition is attributed to the loss of the centre of the protein structure at the hinge, and the second transition is attributed to the denaturation of the three individual Fab and Fc regions. Both glycosylated IgG1 and IgG4 showed higher first transition temperatures than their deglycosylated forms. We conclude that glycan removal significantly perturbs the classic IgG antibody solution structure to become more elongated and less stable.

Understanding the mechanism of a lead interleukin-11 signalling inhibitor

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The multifunctional cytokine interleukin (IL) 11 has recently been shown to have roles in several diseases, including gastrointestinal cancer, where IL-11 signaling has been shown to be a therapeutic target. We have shown that IL-11 forms a hexameric signaling complex with its two receptors, the IL11 specific IL-11Rα, and the shared receptor gp130. Preventing the assembly of the IL-11 signalling complex is a potential strategy for IL-11 signalling inhibition.

We are interested in the structural mechanism of an antagonistic mutant of IL-11, IL-11 Mutein. IL-11 Mutein comprises a mutation in a gp130 binding site (IL-11_{W147A}) which was proposed to reduce the assembly of the hexameric signalling complex, and a second set of mutations (IL-11_{PAIDY}), which were thought to increase affinity for IL-11Rα, enabling Mutein to compete with IL11 for IL-11Rα. We have shown that IL-11 and IL-11 Mutein have similar affinity for IL-11Rα. We have further shown *in vitro*, that IL-11 Mutein abolishes IL-11 signalling, while IL-11_{W168A} permits limited IL-11 signalling, implying that the IL-11_{PAIDY} mutations further decrease affinity for gp130 over IL-11_{W147A}. To understand the structural basis of Mutein antagonism, we have solved the crystal structure of IL-11, IL-11 Mutein and IL-11_{W147A}. These structures show that a loop in the vicinity of a gp130 binding site is displaced in IL11 Mutein compared to IL-11_{W147A}, suggesting that alteration of loop dynamics form the structural basis for IL-11 Mutein antagonism. We are currently conducting molecular dynamics (MD) simulations of IL-11 and IL-11 mutants, to understand the nature of loop dynamics in both proteins.

Poster abstracts

Analytical ultracentrifugation studies of conglutin gamma from *Lupinus angustifolius* seed: Analysis of pH-dependent association-dissociation equilibrium between a monomeric form and an oligomeric assembly (Poster #001)

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Conglutin gamma from lupin is capable of reducing glycaemia in mammals to the levels comparable to those obtained with metformin, a widely used hypoglycaemic drug [1]. Unfortunately, no physiological role and no detailed mechanism of action of gamma-conglutin in the therapy of diabetes have been described as yet.

The protein isolated from *L. angustifolius* (narrow-leaved lupin) seed has a number of unusual properties which include: (i) binding of divalent metal ions, (ii) insensitivity to pancreatin proteolysis, (iii) ability to bind to insulin, (iv) formation of a static complex with flavonoids [2]. X-ray structure demonstrated that the mature native protein is a homohexamer. Each monomer contains two disulphide linked subunits of about 30 and 17 kDa [3]. However, in solution the protein is in a pH-dependent association dissociation equilibrium between a monomeric unit and an oligomeric assembly. In order to get deeper insight into structural changes occurring during the pH-dependent oligomerisation of conglutin gamma, the analytical ultracentrifugation studies were performed.

The sedimentation velocity experiments revealed a loss of the oligomeric form occurring with pH lowering and with concentration decreasing. In pH 7.5 dominates specie with sedimentation coefficient $s_{20,w}$~9.2 corresponding most probably to the tetramer. However, in pH 5.5 the equilibrium between the monomer and the oligomeric assembly is observed, while in pH 4.5 the monomeric form ($s_{20,w}$~4.5) significantly prevails. The sedimentation equilibrium data obtained in pH 7.5 and analyzed by the SedFit program suggest that the oligomer dissociates into monomers. However, none of the models, neither with, nor without intermediate steps, correctly described the data.

We conclude that conglutin gamma belongs to the class of the non-obligate homooligomers and can exist in solution in the pH-dependent oligomeric form, with the assembly dominating in neutral pH and the monomeric form in acidic pH.

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Binding of the axon guidance cue Netrin-1 to heparin oligosaccharides (Poster #002)

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Netrin-1 is a vertebrate axon guidance cue and a trophic factor for the dependence receptor *Deleted in Colorectal Cancer* (DCC). Netrin-1 is secreted into the extracellular matrix (ECM). Interaction with its receptor DCC on the axon surface of central or peripheral neurons leads to chemoactraction of the neuron's growth cone. A chemoattractive axon guidance response is also induced by interaction of Netrin-1 with the related receptor neogenin (NEO1). Wheres DCC is only present on neurons and intestinal cells, neogenin is expressed in a wide range of other tissues. If another receptor, UNC5, is present on the neuron, a triple complex between Netrin-1, DCC and UNC5 forms that reverses chemoattraction into chemorepulsion.

In the intestine, if DCC is not associated with Netrin-1 ligand, the receptor triggers apoptosis. Mutation of DCC or its loss leads to the proliferation of tumors and causes colorectal cancer.

Netrin-1 binds to heparan sulfate which is present in the ECM in form of glycosaminoglycans (GAG) which are long unbranched polymers of amino and uronic sugars. GAG polymers can also be displayed by proteoglycans such as glypicans and syndecans on the cell surface. Anchoring of Netrin-1 to GAGs within the ECM it thought to provide localized gradients of cues leading the growing axon on its way.

In this work we explored the binding Netrin-1 to heparin oligosaccharides of various length using sedimentation velocity experiments in the analytical ultracentrifuge, SEC coupled multiangle light scattering, SEC coupled small angle X-ray scattering, mass photometry and other biophysical methods.

Discovery, Structural Reassignment, and Pharmacological Application of Tetraindole Derivatives as Structurally Novel Histone Deacetylase Inhibitors (Poster #003)

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In pre-menopausal female patients, triple negative breast cancers (TNBCs) occur frequently. The treatments available against TNBC are surgery, radiation and chemotherapy in clinic practice. Even though new molecular targeted agents are now being developed, they are still premature in application of clinical use. Therefore, it is highly demanded to explore the effective antitumor agents against TNBCs. In this study, a series of acyclic tetraindole mimics of CTet, a metabolites of indole-3-carbinol in dietary vegetables, were discovered and evaluated.

Development of selective histone deacetylase inhibitors is attracted attention recently due to the drawbacks of these FDA-approved HDAC drugs displaying serious side effects and cardiotoxicity. In this study, we discovered that a benzene-type tetraindole exhibits selective inhibitory property against histone deacetylase 6 (HDAC6) followed by an increase in the expression of miR-200c. Optimization of the hit compound, in turn, generated a series of tetraindoles. Among these compounds, one 5-hydroxy tetraindole was identified as a potential strong lead compound with IC50 values falling in the nanomolar range 0.24 ~ 2.84 micromolar for 12 different cancer cell line. This activity involves G2/M phase arrest of the cell cycle against breast adenocarcinoma (MCF 7 and MDA-MB-231) cells. Complete mechanism of apoptosis is under investigation. Molecular interactions between small molecule (5-hydroxy tetraindole) and HDAC 6 will be studied using Isothermal Titration Calorimetry (ITC) and Analytical Ultracentrifugation (AUC).

Measuring lipid nanoparticle cargo loading with integral partial specific volume distributions
(Poster #004)

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Determining the amount of drug that is loaded into a lipid nanoparticle (LNP) is of utmost importance for many biopharma applications. This question presents challenges for techniques such as dynamic or static light scattering, size exclusion chromatography, field-flow fractionation, cryo-electron microscopy and related techniques which have difficulty to distinguish between particles that are empty, partially or fully loaded with cargo since their overall size and shape may not be proportionally changing with the load. Analytical ultracentrifugation provides high-resolution bulk separation with excellent statistical certainty by using standard sedimentation velocity experiments (SVEs) to determine the sedimentation and diffusion coefficients, and partial concentrations of the solutes present in the sample. However, in order to derive loading information, these parameters alone are not sufficient. A discrimination based on particle density is also needed to distinguish the loading state of nanoparticles. This information can be obtained from the partial specific volume (PSV) of the solute. If this is known, the particles molar mass and anisotropy can be also derived. Standard analysis protocols assume that the PSV is constant for all particles, which is only valid for some systems. For other systems a PSV or PSV distributions must be explicitly measured. A robust approach for obtaining the value of the PSV in aqueous buffers is to perform density matching experiments(1). Here, the density of the buffer is modulated and SVEs are repeated in different ratios of D2O and H2O, and changes in the sedimentation coefficients are monitored as a function of buoyancy. Each SVE replicate performed at different D2O:H2O ratios was used to generate a g(s) distribution using standard procedures in UltraScan(2). g(s) distributions were integrated to obtain normalized G(s) distributions, and equivalent boundary fractions were extrapolated to s=0, generating a PSV distribution. Corresponding s-values and diffusion coefficients from equivalent boundary fractions can now be used to calculate reliable molar mass, anisotropy, hydrodynamic radius and density distributions for the entire sample. Using this information, the loading of LNPs is now uniquely defined.

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Phase Separation of Binary Mixtures Induced by Weak Centrifugation (Poster #005)

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We use the model system ethanol-dodecane. This binary system has a LCSCT slightly above room temperature. We investigate the behaviour of samples placed above and below the cloud temperature in the single phase when still located in the single phase.

We show that the temperature of phase separation above the miscibility gap can move by several degrees under centrifugation conditions (5000 rpm, which corresponds to gravitational fields of to 1000 g). Moreover, several degrees above the phase transition, i.e. in the one-phase region, strong gradients of ethanol concentration occur. The apparent molar mass of ethanol is of the order of hundred times the real value, which shows a significant degree of aggregation of ethanol molecules. These gradients have no influence on the distribution gradient of solutes such as dyes like Nile red. As a general rule, concentration profiles can be induced equivalently by temperature decrease or centrifugal field increase. The centrifugal field is calibrated, because rotational speeds and molecular volumes are known. This gives a direct access to molecular forces in complex fluids.

It is moreover observed that the interphase formed upon centrifugation appears not as the commonly observed sharp meniscus, but as a relatively wide turbidity zone separating homogenous fluids.

All these effects can be qualitatively understood and explained using Flory-Huggins solution theory. Moreover, the same approach of Jean Perrin allows for a self-consistent definition of the Flory effective volume, and hence an explicit calculation of the total free energy per unit volume in kJ/mole.

Structure and function of a signaling competent Reelin construct (Poster #006)

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The outermost subsection of the brain, the neocortex, is a highly organized and meticulously layered structure. Crucial for our evolution as a species, it is involved in higher order cognitive functions. Its discrete lamination is dependent, in part, on the secreted protein Reelin. Reelin abnormalities cause several diseases. Patients with rare homozygous mutations of the RELN gene present with lissencephaly and cerebellar hypoplasia, while altered levels of Reelin mRNA or protein are associated with neurological diseases, including autism spectrum disorders, schizophrenia, and depression.

Reelin is a glycoprotein with a MW of ~400kDa that binds to the very low density lipoprotein receptor (VLDLR) and the apolipoprotein E receptor 2 (ApoER2). Reelin is a dimer with eight repeat domains that undergoes proteolytic cleavage generating N-terminal, central, and C-terminal fragments. The central dimeric fragment (Repeats 3-6) is required for initiation of the Reelin signal cascade, whereas a monomeric Reelin mutant binds its receptors but does not signal.

Here, we present data from a variety of biophysical and structural techniques providing preliminary details on a dimeric structure of the central fragment of Reelin in comparison with its monomeric counterpart. Using cryo-EM, we show that dimeric, signaling competent Reelin adopts an X-shaped configuration, linked in a fashion consistent with its known dimerization site (C2101). SAXS supports the size and shape of Reelin seen in the cryo-EM data, and size exclusion chromatography along with western blotting provides evidence of the formation of a complex between Reelin and VLDLR. We also present a biological assay to study VLDLR and ApoER2 activation after various Reelin isoform binding.

Taken together, our data show an initial characterization of the structure of a signaling competent Reelin construct, and we present a functional assay to analyze why signal activation is dependent on the oligomeric state of Reelin.

Studies of interacting protein systems using multi-wavelength AUC (Poster #007)

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Multi-wavelength AUC (MW-AUC) allows for the spectral separation of solutes based on their unique chromophores, along with their hydrodynamic separation (1). When the solutes intrinsic molar extinction spectra are known a priori, they can be used to deconvolute the MW-AUC data. This deconvolution allows for the hydrodynamic signal to be optically separated into multiple solutes, each with a different chromophore. Utilizing the benefits of MW-AUC will allow for the quantitative characterization of a compounds stoichiometry, hydrodynamic radius and a more in depth understanding of solute binding than seen in typical sedimentation velocity experiments. MW-AUC experiments can elucidate the stoichiometry of many different systems including, systems where fluorescently labeled fusion proteins interact with an unlabeled proteins or proteins labeled with a fluorophore or fluorescent protein containing a distinct and different chromophore. MW-AUC combines the benefits of two orthogonal separation methods: 1. the separation of proteins based on their hydrodynamic and 2. based on their spectral properties. This can be extremely useful for systems where the hydrodynamic properties are identical. This has been shown using teal fluorescent protein (TFP1), mPapaya fluorescent protein (mPapaya) and ultramarine fluorescent protein (UFP). We have shown that mPapaya, TFP and UFP can be 100% baseline resolved when using MW-AUC because of their distinct absorbance spectrum, this allows for accurate determination of the partial concentration of each protein in the solution. In this case, mPapaya and TFP have the same sedimentation coefficient, but UFP sediments faster since it exists as a dimer. Results show that each molecule can be well resolved despite overlapping spectra and hydrodynamics.

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The structure and function of novel *Coxiella burnetii* effector proteins (Poster #008)

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Coxiella burnetii is an obligate intracellular pathogen that causes Q fever in humans and animals. Essential to the intracellular success of *C. burnetii* is the establishment of the unique, replicative, autolysosomal niche termed the Coxiella containing vacuole (CCV). *Coxiella burnetii* utilises an arsenal of over 130 type IV effector proteins (T4Es) some of which modulate host cell trafficking pathways allowing for the formation of the CCV. Cig57 (Cbu1751), Cbu1752, and Cbu1754 are three novel Coxiella T4Es implicated in CCV biogenesis through the subversion of host clathrin-mediated transport. Cig57 interacts with the host protein FCHO2, a nucleator of clathrin-mediated transport. A Cig57/FCHO2 interaction re-routes clathrin to the CCV with unknown effect, and was thought to be mediated by endocytic sorting motifs present on Cig57. Cbu1752 and Cbu1754 are hypothesised to act synergistically with Cig57, based on the ability of Cig57, Cbu1752, and Cbu1754 to co-immunoprecipitate. Although Cig57, Cbu1752, and Cbu1754 have been identified as T4Es implicated in CCV expansion, their mechanistic function and relationship with each other and the clathrin mediated transport system as a whole remains unknown.

Using a combination of analytical ultracentrifugation (AUC) and small angle x-ray scattering (SAXS) experiments, the basic structural characteristics of Cig57, Cbu1752, and Cbu1754 were determined. Cig57 and Cbu1754 are monomeric and roughly globular in solution, whereas Cbu1752 adopted a more extended conformation and displayed low levels of self-association. The crystal structure of the Cig57 central domain (residues 178-307) was solved with a resolution of 1.75 Å. The central domain of Cig57 adopts a 4-helix bundle, which in conjunction with small angle scattering data was found to form an inner groove on the surface of Cig57. Additionally, two previously hypothetical endocytic sorting motifs were found to form the hydrophobic core of Cig57, ruling out the possibility that these motifs have biological relevance in binding FCHO2. This work is the first to structurally characterise any Coxiella protein, leading to a better understanding of novel effector protein function and clathrin mediated transport.

UltraScan GMP software for Biopharma (Poster #009)

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Recent advances in instrumentation have moved analytical ultracentrifugation (AUC) closer to a possible validation of this technique for a Good Manufacturing Practices (GMP) environment. In order for AUC to be validated for a GMP environment, stringent requirements need to be satisfied. These requirements extend to multiple regulatory aspects, covering instrument hardware, data handling and software for data acquisition and data analysis, process control, audit trails and automation. Here we review the requirements for GMP validation and illustrate solutions that can address a subset of these requirements focused on the operation and data handling of the latest analytical ultracentrifuge, the Optima AUC by Beckman Coulter.

Nanobody fusion constructs for NK cell immunotherapy (Poster #010)

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Natural killer (NK) cells are an important part of the innate immunity. They exhibit cytotoxic response against stressed, infected or malignantly transformed cells. The activation of this process is realized through binding of specific surface ligands of harmed cells to activating receptors of NK cells. But tumour cells strive to escape the immune system surveillance. Thus, the reconstitution of the cytotoxic recognition could be an interesting target of tumour immunotherapy.

We have prepared bivalent fusion proteins able to recognize and bind both tumour cells, and NK cells. The fusion proteins consist of VHH nanobody targeting specific tumour cell marker HER2, and of extracellular domain of MICA, a ligand for the activating NK cell receptor NKG2D. Such approach allows two possible designs of the fusion protein: the activating ligand positioned on N-terminus and the VHH nanobody on C-terminus of the protein or the inverse arrangement. The *in vitro* binding studies have revealed that the arrangement plays an important role in the binding capacity of both parts of the fusion protein. The position of MICA on the C-terminus is favourable for NK cell activating receptor binding, whereas the position of antiHER2 nanobody on the N-terminus is advantageous for targeting the tumour marker on the cell surface.
The same approach was used for construction of another fusion protein containing B7-H6, a ligand for the activating NK cell receptor ligand NKp30, and its binding capacity was characterized as well. The prepared fusion proteins will be further used with tumour and NK cell lines where their potential to establish the cytotoxic response will be evaluated.

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The Interactions of Oleosins within Complex Food Systems (Poster #011)

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Seeds need fuel to germinate and grow into a plant. Oil bodies are micelles that hold the triacetyl glycerides (TAGs) or "fat" in seeds. They are comprised of a phospholipid monolayer encasing the TAGs inside. Oleosins are proteins that are imbedded in the membrane of the oil body. The terminals of the protein are hydrophilic and sit on the outside of the membrane along with the phospholipid heads. There is a central hydrophobic domain that inserts into the membrane anchoring the protein in the membrane. The hydrophobic domain contains a highly-preserved proline knot motif that creates a hairpin turn and brings the amino acid chain around to bring the terminal to the top of the membrane. I am working to understand the interaction between Oleosins, and any interactions that may take place between the oleosins and the phospholipid membrane, and the TAGs on the inside of the oil body. This will include Analytical Ultracentrifugation experiments. A structural analysis will also be done to further map the protein. Finally, I will be doing a digestive analysis to determine if this plant-based protein can be used in lab-based food systems.

Substrate specificity in SiaT mutants (Poster #012)

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Staphylococcus aureus and *Proteus mirabilis* are pathogenic bacteria that are resistant to many clinical antibiotics and the identification of new inhibitory compounds is a priority for human health. Our approach to this challenge is to investigate the proteins of the sialic acid pathway. Sialic acids are scavenged by *S. aureus* and *P. mirabilis* from mammalian hosts as carbon and nitrogen sources, but also to be used on their cell surfaces in order to evade the host immune system. Our focus here is on the sialic acid transporter SiaT, a membrane protein that can be directly targeted by inhibitory compounds.

N-Acetylneuraminate and *N*-glycolylneuraminate are the most abundant sialic acids, with *S. aureus* SiaT having a higher affinity for *N*-glycolylneuraminate, whereas the homologous SiaT from *P. mirabilis* has a higher affinity for *N*-acetylneuraminate. However, the active sites of these two homologues only differ in three amino acid residues. Examination of the X-ray crystal structure of *P. mirabilis* SiaT, and the subsequently modelled *S. aureus* structure, suggests that these three residues may be entirely responsible for the alternative substrate specificity these two SiaT homologues display.

We are investigating substrate preference by engineering two SiaT triple mutants; the *S. aureus* and *P. mirabilis* SiaT, each with the three active residues substituted to the alternative configuration. The characteristics of these will then be examined by isothermal titration calorimetry, microscale thermophoresis, whole cell growth assays and X-ray crystallography.

Preliminary characterisation of a sugar transporter complex by analytical ultracentrifugation
(Poster #013)

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Tripartite ATP-independent Periplasmic transporters (TRAPs) are comprised of a membrane embedded secondary transporter and a soluble binding partner. We are interested in TRAPs that import sialic acid across the periplasmic membrane in gram-negative bacteria. These transporters, termed SiaPQM, are not well characterised, and details on the interaction between the membrane component and the soluble component are currently unknown. We are characterising this interaction using sedimentation velocity AUC, with both absorbance and fluorescence detection of the sedimenting species. By measuring the dissociation constant (K_d) of the transporter complex under varying conditions we are able to characterise the binding affinity of the complex and determine the effect that the substrate and transport ions have on this affinity.

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